INFECTION LEVELS OF TWO ASCARIDOID NEMATODES (*ANISAKIS SIMPLEX* AND *PSEUDOTERRANOVA DECIPIENS*) IN ATLANTIC COD (*GADUS MORHUA*) OFF NEWFOUNDLAND AND LABRADOR, AND ON THE FLEMISH CAP

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ABSTRACT

Parasitic nematodes infecting the flesh of commercially important fish species pose an aesthetic and economic problem for the fishing industry. They also have potential health implications as they may cause disease in humans. The musculature of 811 Atlantic cod (*Gadus morhua*) from five cod stocks in the Northwest Atlantic off Newfoundland and Labrador, and on the Flemish Cap, were examined for two ascaridoid nematodes, *Anisakis simplex* sensu lato and *Pseudoterranova decipiens* sensu lato. I evaluated the distribution of these nematodes within the musculature of Atlantic cod, and investigated whether prevalence, abundance, or density varied among cod stocks or in relation to fish length. I used similar examination techniques to previous studies conducted during the 1940-50s and the 1980s, and compared infection levels to these studies. In all areas, prevalence and abundance of *A. simplex* s.l. increased substantially relative to historical studies. The highest infection levels of *A. simplex* s.l. were observed in fish from Northwest Atlantic Fisheries Organization (NAFO) Division 3M. The results are consistent with a possible increase in abundance of some cetaceans, the definitive hosts of *A. simplex* s.l.; although population trends of most cetaceans are not well known. In most areas, infection levels of *P. decipiens* s.l. also increased since the 1980s. The area with the highest abundance of *P. decipiens* s.l. was the west coast of Newfoundland (Divisions 3Pn4R). Greater abundances could be related to an increase in abundance of grey seals (*Halichoerus grypus*) that serve as definitive hosts. Major changes in marine food webs following the collapse of groundfish stocks in the late 1980s and early 1990s, as well as

increasing ocean temperatures, may also have influenced the population dynamics of these parasitic nematodes.

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Table of Contents

List of Tables

List of Figures

List of Appendices

1 General Introduction

Atlantic cod (*Gadus morhua*; referred to as "cod" in this thesis) is a demersal gadoid inhabiting the North Atlantic, primarily on continental shelfs. As a large predator at a high trophic level, it plays an important role in the ecosystem (Link et al. 2009). In the Northwest Atlantic around Newfoundland and Labrador, cod is managed as five separate stocks; the northern Labrador cod in Northwest Atlantic Fisheries Organization (NAFO) Divisions 2GH, the northern cod stock in Divisions 2J3KL, the southern Grand Bank stock in Divisions 3NO, the southern Newfoundland stock in Subdivision 3Ps, and the northern Gulf of St. Lawrence stock in Divisions 3Pn4RS. A fifth stock, the Flemish Cap stock in Division 3M, lies outside of Canadian waters (Fig. 1.1).

Cod have been harvested for centuries, and the fishery for this species was once the largest in Atlantic Canada (Scott and Scott 1988; Rose 2007). Following the collapse of many stocks in the late 1980s and early 1900s (Myers et al. 1996), many of the fisheries were placed under moratoria with the expectation that the populations would recover rapidly. Some fisheries have subsequently reopened although none have recovered to pre-moratorium levels.

A common problem when wild animals such as Atlantic cod are used for human consumption is the presence of parasites. They can occur in all wild animals and may be both unsightly and pathogenic to humans. Marine parasites are often generalists, capable of infecting multiple species of invertebrate, fish, and mammals (Marcogliese 2002). Marine fish can serve either as definitive hosts (supporting the sexually reproducing life stage) or intermediate hosts (harbouring the parasite for at least part of its development;

Marcogliese 2002). Many commercially exploited marine organisms can become infected with parasites which can be a problem for the seafood industry (McClelland 2002).

Atlantic cod serve as the definitive or intermediate host for many parasitic species (at least 107), most occurring in the viscera (Margolis and Arthur 1979; McDonald and Margolis 1995; Hemmingsen and MacKenzie 2001). The varied parasite fauna of Atlantic cod may be due in part to their generalist diet and wide distribution. This puts them in contact with many species of fish, invertebrates and other prey that may be host to numerous parasite species.

The fishing industry has expressed concern regarding the abundance of parasitic anasakid nematodes in the musculature of Atlantic cod. Nematodes in the flesh decrease the quality of fish products, are costly to remove, and are unappealing to consumers (Jenks et al. 1996; McClelland 2002). Many nematodes also pose a health concern as humans can become infected potentially resulting in zoonotic diseases. The parasites causing the most concern for the industry in Atlantic Canada, as well as being the most abundant in the flesh of cod, are two species complexes of ascaridoid nematodes, *Anisakis simplex* sensu lato and *Pseudoterranova decipiens* sensu lato, commonly known as whale worm and seal worm, respectively (Fig. 1.2).

1.1 Ecology and life cycle of *A. simplex*

Anisakis simplex s.l. is a complex of species, with *A. simplex* sensu stricto being the primary species infecting marine fishes around Newfoundland and Labrador (Brattey and Bishop 1992; Mattiucci et al. 1997). This anisakid nematode has a broad distribution, infecting many species in most of the world's oceans, and is commonly found in a

number of commercially important marine fishes. In Atlantic Canada, hosts include Atlantic cod (Templeman et al. 1957; Chandra and Khan 1988; Brattey and Bishop 1992; Boily and Marcogliese 1995; McClelland et al. 2011; Mehrdana et al. 2014), American plaice (*Hippoglossoides platessoides*; Boily and Marcogliese 1995), and pelagic fishes such as Atlantic herring (*Clupea harengus*; McGladdery 1986) and capelin (*Mallotus villosus*; Hays et al. 1998).

Anisakis simplex s.l. typically follows a pelagic life cycle where the definitive hosts are various marine mammals, primarily cetaceans, such as belugas (*Delphinapterus leucas;* Measures et al. 1995) and harbour porpoises (*Phocoena phocoena*; Ugland et al. 2004). Mature nematodes live in the stomach of the definitive host and release eggs that are expelled during defecation. The eggs become embryonated in seawater and sink to the sediment on the ocean floor where they moult within the egg before hatching into the third developmental stage (L3) larvae (Measures and Hong 1995; Køie et al. 1995; Audicana and Kennedy 2008). Subsequently, L3 larvae consumed by small crustaceans are released from their cuticle and can encyst within these intermediate hosts (Køie et al. 1995). These obligate intermediate hosts (a host which the parasite must infect to undergo moulting, growth, or a morphological or developmental change) are preyed upon by numerous macroinvertebrates. This then allows *A. simplex* s.l. to become infective to fish species, including cod and herring, which serve as transport or paratenic hosts (where little or no development of the parasite occurs) and the L3s can be transferred to the definitive host during predator-prey interactions (Køie et al. 1995; Hays et al. 1998; Klimpel et al. 2004). Records of cetacean diets off Newfoundland are limited and, although some cetaceans may prey on Atlantic cod, it has not been considered to be an

important prey item for most species (J. Lawson, pers. comm., 19 Feb. 2014). This suggests that Atlantic cod may be a dead end for many L3 larvae as they are not likely to be consumed and therefore transmitted to their definitive hosts in significant numbers.

Most *A. simplex* s.l. larvae are found in the viscera of their fish hosts, but some migrate into the flesh (Brattey and Bishop 1992). Most larvae in the flesh are encysted in the napes (flesh surrounding the body cavity) of the fish, though some are in the fillet (dorsal musculature anteriorly, and both dorsal and ventral musculature posteriorly; Chandra and Khan 1988; Brattey and Bishop 1992).

1.2 Ecology and life cycle of *P. decipiens*

Pseudoterranova decipiens sensu lato (s.l.) also comprises a species complex with three of the five known species occurring in the North Atlantic (Paggi et al. 1991; McClelland 2002). Of these three morphologically similar species, *P. decipiens* sensu stricto (s.str) primarily infects grey seals (*Halichoerus grypus*) which are an important host, and to a lesser extent harbour seals (*Phoca vitulina*) in North Atlantic waters (Paggi et al. 1991; Brattey and Stenson 1993; Brattey and Davidson 1996; Marcogliese et al. 1996). Although hooded seals (*Cystophora cristata*) and harp seals (*Phoca groenlandica*) may become infected, they typically have very low numbers of nematodes and are not considered an important host to *P. decipiens* s.str (Paggi et al. 1991; Brattey and Stenson 1993; Brattey and Davidson 1996; Marcogliese et al. 1996). Most larvae in fishes around Newfoundland were reported to be *P. decipiens* s.str. although some specimens from Labrador represent a second species, *Pseudoterranova bulbosa* (Brattey and Davidson 1996). The adults of *P. bulbosa* occur in bearded seals (*Erignathus barbatus*) and larvae

have been observed in American plaice and Greenland halibut (*Reinhardtius hippoglossoides*; Brattey and Davidson 1996). The larvae of *P. bulbosa* occur in the viscera, not the flesh, and are therefore less problematic for the fishing industry.

In contrast to *A. simplex* s.l., *P. decipiens* s.l. has a more benthic life cycle. Sexually mature *P. decipiens* s.l. reside in the stomach of phocids and the life cycle involves release of nematode eggs along with the feces of the definitive host. The eggs at the first larval developmental stage (L1) undergo a second moult and these second stage larvae (L2) settle to the seabed (McClelland 2002). There is some debate about the number of moults that occur within the egg before hatching into ensheathed larvae (L3) (Køie et al. 1995; McClelland 2002). The larvae are subsequently ingested by various species of invertebrates, such as copepods, and macroinvertebrates such as crustaceans and amphipods, which act as second intermediate hosts (McClelland et al. 1990; Marcogliese 2001b; McClelland 2002). Within this host, the nematode larvae grow to approximately 2-3 mm in length, after which they are capable of infecting fish (McClelland 2002). Fish such as American plaice (Bristow and Berland 1992; Martell and McClelland 1995), yellowtail flounder (*Pleuronectes ferrugineus*; Martell and McClelland 1995), and Atlantic cod (Templeman et al. 1957; Brattey et al. 1990; Hauksson 2011) act as the third intermediate host. Fish are important hosts for *P. decipiens* s.l. larvae as they support parasite growth and, due to their longevity and migration patterns, are also important in the dispersal of the nematode (McClelland 2002).

Grey seals are an important definitive host for *P. decipiens* s.l. in Atlantic Canada (Brattey and Stenson 1993; McClelland 2002). Cod can be a substantial part of grey seal diet, particularly for males during the winter (Hammill et al. 2014), contributing to higher

number of *P. decipiens* s.l. infecting grey seals. Grey seals tend to consume small cod (typically less than 35 cm in body length), though large fish can be eaten (up to 80 cm in body length; Bowen and Harrison 1994; Hammill et al. 2007; Hammill et al. 2014).

Distribution of *P. decipiens* s.l. larvae within fish hosts varies with size of the fish, with larger fish tending to have more *P. decipiens* s.l. larvae, which may be partially a function of diet (McClelland 2002). The numbers of *P. decipiens* s.l. larvae in the napes and body cavity tend to increase with fork length while in smaller fish, most larvae are in the fillets (McClelland 2002; Brattey et al. 1990). Nematodes may survive multiple transmissions among fish hosts (Burt et al. 1990), meaning they can accumulate more rapidly in larger individuals when they become piscivorous. However, as for *A. simplex* s.l., *P. decipiens* s.l. larva may lose vigour as they pass through multiple hosts which may increase their risk of being lost to the fish's immune system (Jensen 1997; McClelland 2002).

1.3 Factors influencing parasite abundance

Many factors influence the abundance of marine parasites. For example, generalist parasitic species that are able to infect a wide range of hosts have a greater likelihood of survival and reaching their definitive host (Marcogliese 2002). An increased number of infected hosts combined with high larval longevity within intermediate hosts increase the distribution of the nematode (Marcogliese 2002). *Anisakis simplex* s.l. infects various host species over diverse habitats and depths, but are most likely to complete their life cycle by infecting species in the pelagic zone (Kuhn et al. 2013). *Pseudoterranova decipiens* s.l. also infects a broad range of species in various habitats that influence its distribution

in the oceans, although most of its fish hosts tend to be benthic rather than pelagic (McClelland 2002).

Northwest Atlantic ecosystems have undergone major changes during recent decades (Worm and Myers 2003), particularly in the region off Newfoundland and Labrador. Through a combination of overfishing and poor environmental conditions, Atlantic cod, once a dominant predator in these waters, underwent drastic population declines during the 1980s and early 1990s (Rose 2007; Lilly et al. 2013). Concurrently, capelin, once a highly abundant pelagic forage fish, also underwent drastic declines in numbers in response to environmental change (Buren et al. 2014; DFO 2015). At the same time, northern shrimp and snow crab increased, leading to an invertebrate dominated system (Worm and Myers 2003). Within these changing ecosystems, it is likely that transmission and abundance of marine parasites also changed (Marcogliese 2001a; 2002).

At the same time, abundance of grey seals, the definitive host of *P. decipiens* s.l., increased from a few thousand animals during the 1960s to over 400,000 in 2016 (DFO 2017b). High parasite abundance in fish have been correlated to this increase in grey seal populations (McClelland 2002; Mehrdana et al. 2014; Lunneryd et al. 2014).

Pseudoterranova decipiens s.l. is abundant in fish that are found close to grey or harbour seal haulout sites or breeding colonies (Jensen and Idås 1992; Lunneryd et al. 2001; Hauksson 2002; Hauksson 2011). The highest infection rates of nematode larvae in fish are found near areas densely populated with grey seals (Bowen 1990). Throughout the biotic changes described above, *A. simplex* s.l. and *P. decipiens* s.l. have persisted and remained widespread, probably in part because of their low host specificity.

Abiotic changes in the environment may also affect nematode abundance. For example, bottom temperatures must be suitable for the development of the hatching nematodes and their transmission to intermediate hosts (McClelland 2002). *Pseudoterranova decipiens* s.l. eggs may die if exposed to bottom temperatures below 0°C and, after warming trends occur, nematodes in cod increase in abundance (McClelland et al. 2011). *Anisakis simplex* s.l. is also adversely affected by decreasing bottom temperatures; hatching times greatly increased from a few weeks at 8°C to months at 2°C (Brattey and Clark 1992). With the increasing trend seen in sea-bottom temperatures in many areas around Newfoundland and Labrador, eggs of both nematode species may start to develop more rapidly (Brattey and Clark 1992; McClelland 2002; Colbourne et al. 2016).

1.4 Effects of parasites on fish hosts

Parasites can have many adverse effects on their hosts. It is possible that parasite load may indirectly impact natural mortality by increasing host suceptibility to predation (DFO 2011; McClelland et al. 2011). *Pseudoterranova decipiens* s.l. damages internal organs while migrating from the stomach to the flesh, which could impair the host's health and lead to an inability to forage efficiently or escape predation (McClelland et al. 2011). Ketones secreted as a by-product of metabolism by larval *P. decipiens* s.l. may act as a local anesthetic, which reduces swimming speed in European smelt (*Osmerus eperlanus*), and adversely affects overall condition which may thereby increase suceptibility to predation (Möller and Klatt 1990; Sprengel and Lüchtenberg 1991). Although, McClelland et al. (2011) found a weak positive relationship of condition of cod

to parasite load, this may have resulted because fish of better condition are likely to be more efficient foragers which would correspondingly increase their intake of parasites.

1.5 Implications for human health

Humans are not suitable hosts for *A. simplex* s.l. or *P. decipiens* s.l., though they may become infected when raw or improperly cooked fish is consumed. However, anisakiasis, a zoonotic disease caused primarily by *A. simplex* s.l., may be contracted if live parasites are eaten (Vaughan et al. 2015). *Pseudoterranova decipiens* s.l. can also cause this disease in humans (Alonso-Gomez et al. 2004; Audicana and Kennedy 2008; Hochberg and Hamer 2010). Proper cooking and freezing techniques eliminate the threat of anisakiasis, but many cases of this disease occur worldwide in regions where raw fish is consumed (Audicana and Kennedy 2008). Finally, anisakid nematodes are also a cause of severe allergic reactions in a few individuals, even after fish has been properly cooked or frozen (Audicana and Kennedy 2008).

1.6 Technological solutions and preventative measures

Currently, candling is the preferred technique utilized for detecting parasitic nematodes in fish musculature. The process involves placing the musculature over a white light table where parasites are revealed as dark spots (Fig. 1.3). However, the procedure is labour-intensive and inefficient, with detection efficiencies of 33-93% (McClelland 2002). Other methods of parasite detection and removal are being developed, such as imaging spectroscopy (Heia et al. 2007), magnetic detection (Choudhury and Bublitz 1994), and sensitive magnetometer detection (Jenks et al. 1996);

nevertheless, candling remains to be the primary method used by most processing plants (McClelland 2002).

1.7 Objectives

Infection levels of larval ascaridoid nematodes in the musculature were previously assessed as part of parasitological surveys of Atlantic cod surrounding Newfoundland and Labrador during 1947-1953 (Templeman et al. 1957) and 1985-1987 (Brattey et al. 1990; Brattey and Bishop 1992). Templeman et al. (1957) reported very low infection levels of *P. decipiens* s.l. and *A. simplex* s.l. in most areas around the province with the highest levels occurring along the west and south coasts of Newfoundland. Brattey et al. (1990) and Brattey and Bishop (1992) found a similar geographic pattern during the 1980s, with abundance and density appearing to have increased among cod along the south coast of Newfoundland since the study by Templeman et al. (1957; cod from western Newfoundland were not examined in the later studies). With some cod stocks off Newfoundland and Labrador showing signs of improvement in recent years (Rose and Rowe 2015; DFO 2017a), there has been a renewed concern about anisakid nematode infection in this species, stressing the importance of acquiring updated information on their occurrence. I examined 811 Atlantic cod collected from five separate stocks surrounding Newfoundland and Labrador during 2012-2013, to quantify infection levels of *A. simplex* s.l. and *P. decipiens* s.l. for comparison with previous studies. I also evaluated the distribution of larval *A. simplex* s.l. and *P. decipiens* s.l. within the musculature of Atlantic cod, and investigated whether prevalence, abundance, or density of these larval nematodes varied among cod stocks or in relation to fish length.

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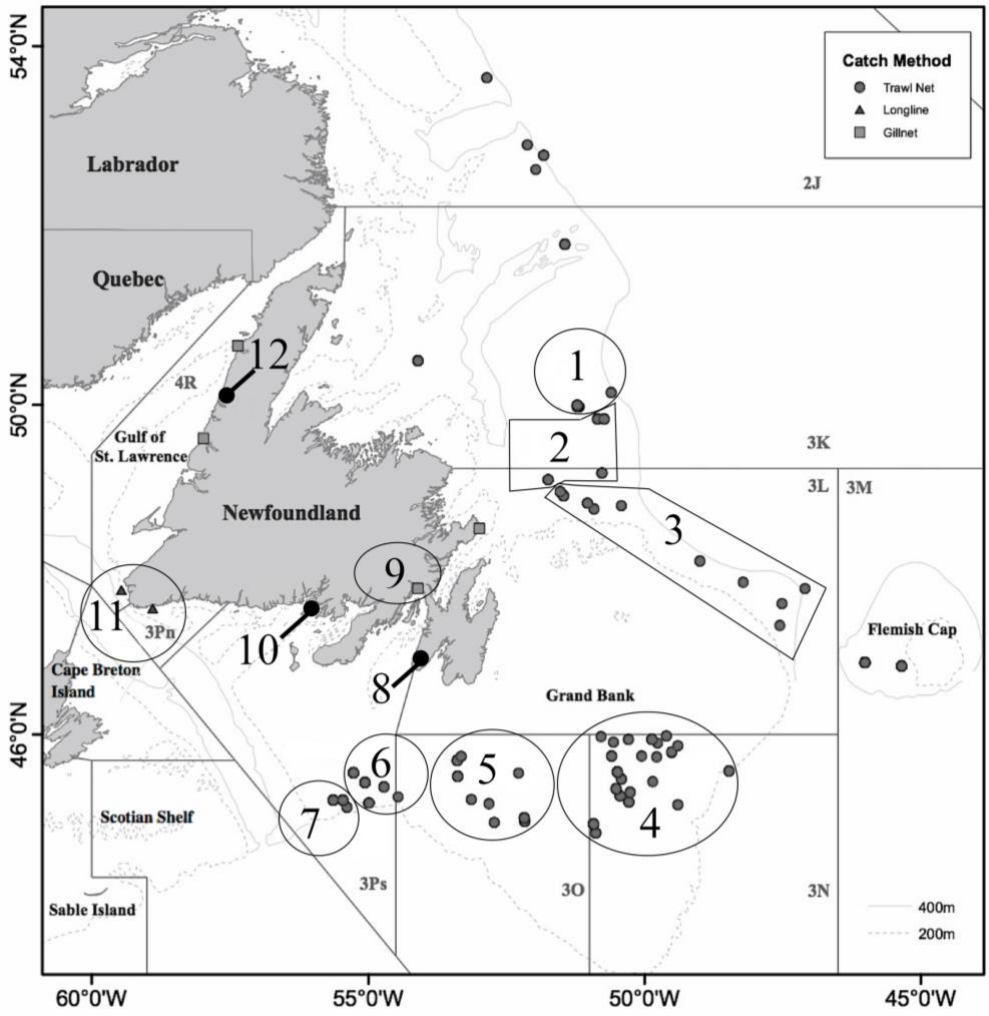

Figure 1.1 Study area indicating NAFO Division boundaries and locations where Atlantic cod (*Gadus morhua*) were sampled during 2012-2013. Numbers within shapes correspond with the samples that were grouped for comparison with Figure 1 of Brattey and Bishop (1992; Appendix B). 1 = Southern Funk Island Bank, 2 = Northeast Newfoundland Shelf, 3 = Grand Bank, 4 = Southeast Shoal of the Grand Bank, 5 = Whale Bank, 6 = Green Bank, 7 = Southern St. Pierre Bank, 8 = Cape St. Mary's, 9 = Placentia Bay, 10 = Pass Island, 11 = Rose Blanche Bank, 12 = Portland Creek. Each point indicates a set where multiple fish were collected (Appendix A).

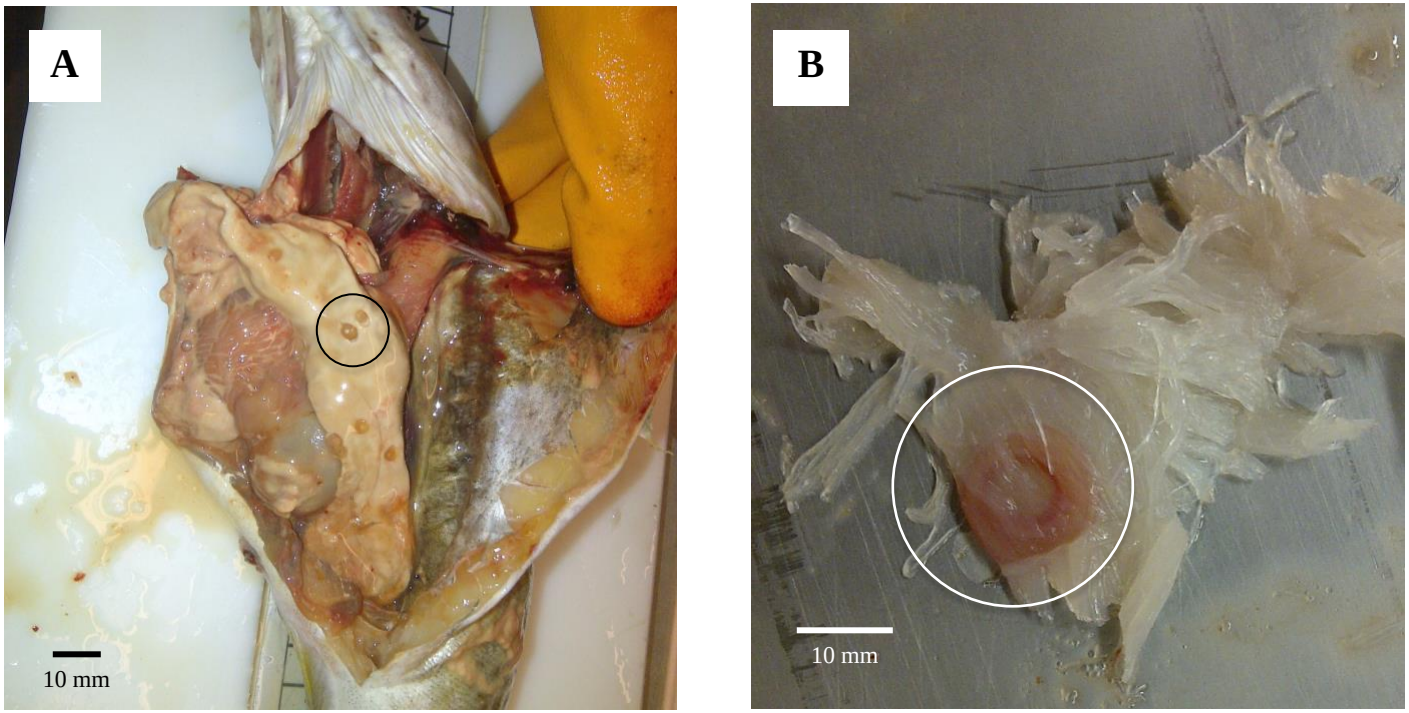

Figure 1.2 A. The viscera of an Atlantic cod (*Gadus morhua)* showing *Anisakis simplex* s.l. encysted in the liver, two nematodes can be seen within the circle. B. Within the circle is an encysted *Pseudoterranova decipiens* s.l. in the flesh of an Atlantic cod. Scales seen in both photographs are approximate.

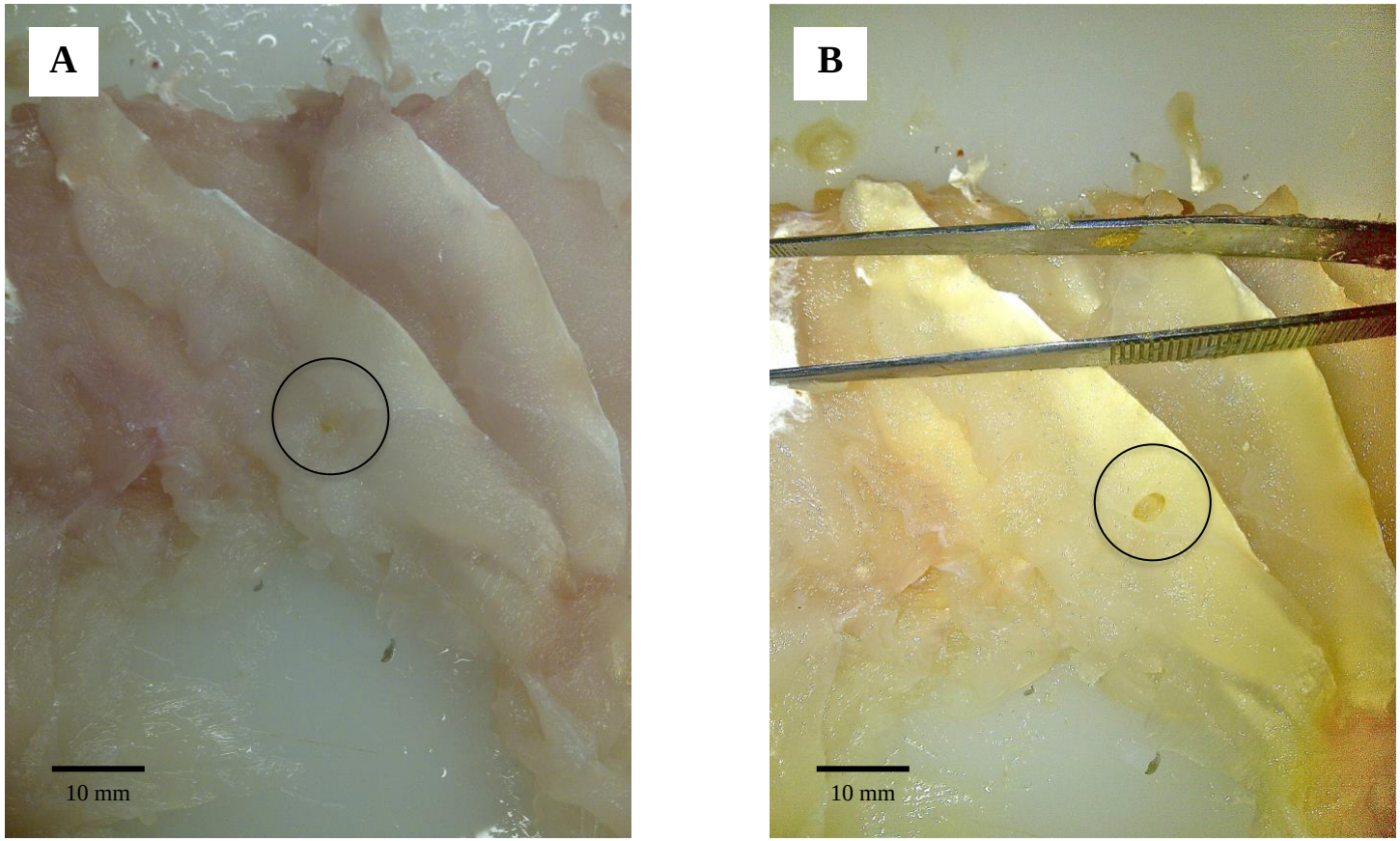

Figure 1.3 A. Fillet portion of Atlantic cod (*Gadus morhua*) on the candling table. B. The same portion of fillet with the candling table turned on. The illumination causes nematodes, including *Anisakis simplex* s.l. (pictured above in the circle), to appear as dark spots and become more easily detectable. Scales seen in both photographs are approximate.

2 Infection levels of larval *Anisakis simplex* (Nematoda: Ascaridoidea) in the musculature of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador and on the Flemish Cap

2.1 Abstract

Parasitic nematodes infecting the flesh of commercially important fish species pose aesthetic, economic and public health problems. For example, the presence of *Anisakis simplex* sensu lato (Nematode: Ascaridoidea) in the flesh of fish may cause anisakiasis, a zoonotic disease contracted by ingesting live parasites when fish are eaten raw or have been improperly cooked. The musculature of 811 Atlantic cod (*Gadus morhua)* from five cod stocks in the Northwest Atlantic off Newfoundland and Labrador, and on the Flemish Cap were examined for *A. simplex* s.l. I evaluated the distribution of this nematode within the musculature of Atlantic cod, and investigated whether prevalence, abundance, or density varied among cod stocks or in relation to fish length. This study used similar examination techniques to previous studies conducted in the same general areas during 1947-1953 and 1985-1987. Compared to these earlier studies, infection levels of *A. simplex* s.l. in the musculature of cod have increased substantially among most areas and length classes of cod. The highest abundance of *A. simplex* s.l. observed was on the Flemish Cap (Northwest Atlantic Fisheries Organization (NAFO) Division 3M). Reasons for the increased infection levels of *A. simplex* in cod are difficult to discern. It is not known if this increase is associated with an increase in cetacean abundance, the definitive host of *A. simplex* s.l., as population trends of most species are unknown. Major changes

in marine food webs following the collapse of groundfish stocks in the late 1980s and early 1990s, as well as increasing ocean temperatures, may also have influenced the population dynamics of this parasitic nematode. This study also emphasizes the importance of evaluating and reporting detection efficiencies when examining the flesh of fish for nematodes so that infection levels can be compared between studies.

2.2 Introduction

Anisakis simplex sensu lato, commonly known as whaleworm, is a parasitic nematode with a complex life cycle. *Anisakis simplex* s.l. appears to be broadly distributed having been isolated from many species of invertebrates, fish, and cetaceans (Klimpel et al. 2010). *Anisakis simplex* s.l. is a complex of species, with *A. simplex* sensu stricto being the primary species infecting marine fishes around Newfoundland and Labrador (Brattey and Bishop 1992; Mattiucci et al. 1997). Adults live in the digestive tract of marine mammals, primarily cetaceans, with invertebrates such as euphausiids serving as intermediate hosts and a variety of fishes and squids included as paratenic hosts (Hays et al. 1998a; Strømnes and Andersen 1998; Abollo et al. 2001; Choi et al. 2011). This parasite has received considerable scientific attention, as third-stage (L3) larvae have been reported from commercially important marine fishes including capelin (*Mallotus villosus*; Palsson and Beverley-Burton 1984; Hays et al. 1998b), Atlantic herring (*Clupea harengus*; Parsons and Hodder 1971; McGladdery 1986; Hays et al. 1998b), Atlantic salmon (*Salmo salar*; Senos et al. 2013), Atlantic cod (Templeman et al. 1957; Chandra and Khan 1988; Brattey and Bishop 1992; Boily and Marcogliese 1995;

McClelland et al. 2011; Mehrdana et al. 2014), and American plaice (*Hippoglossoides platessoides*; McClelland et al. 1983a; 1983b; 1985; Boily and Marcogliese 1995).

Anisakis simplex s.l. poses a significant economic issue for the fishing industry as the detection and removal of nematodes from fish products reduce their market value and increase the cost of processing (Malouf 1986; Jenks et al. 1996; McClelland 2002). *Aniaskis simplex* s.l. can infect humans, resulting in the disease anisakiasis (Audicana and Kennedy 2008), and so also presents a public health concern. Although larvae can be killed by thorough freezing or cooking (Margolis 1977; McClelland 2002), the number of cases of anisakiasis worldwide is rising, possibly due to changes in parasite abundance, greater awareness of the disease, or changing dietary habits which include increased consumption of raw or lightly cooked seafood (Audicana et al. 2002; Hochberg and Hamer 2010). *Anisakis simplex* s.l. allergens are resistant to cooking and freezing, and many cases of allergic reactions to dead nematodes have been reported (Moneo et al. 2000; Audicana et al. 2002; Daschner and Pascual 2005; Hochberg and Hamer 2010). In this regard, there is a need for information on the occurrence of *A. simplex* s.l. within and among fish stocks harvested for human consumption.

Northwest Atlantic ecosystems have undergone major changes during the last three decades (Worm and Myers 2003) and perhaps nowhere have these changes been as dramatic as in the region off Newfoundland and Labrador. Atlantic cod, which were once the dominant large predatory fish in these waters, are estimated to have declined more than 99% in some areas since the 1960s (COSEWIC 2010) through a combination of over-fishing and poor environmental conditions during the late 1980s and early 1990s (Rose 2007; Lilly et al. 2013). Concomitant with the collapse of Atlantic cod in the early

1990s was an equally significant collapse in capelin (Buren et al. 2014), the most important forage fish from Labrador to southern Grand Banks and an important prey species for many fishes, birds, and marine mammals (Scott and Scott 1988; DFO 2015a). Within these altered ecosystems, it is likely that transmission, abundance, and diversity of marine parasites have also changed (Marcogliese 2001; 2002), particularly those transmitted through predator-prey interactions.

In the Northwest Atlantic around Newfoundland and Labrador, cod is managed as five separate stocks; the northern Labrador cod in Northwest Atlantic Fisheries Organization (NAFO) Divisions 2GH, the northern cod stock in Divisions 2J3KL, the southern Grand Bank stock in Divisions 3NO, the southern Newfoundland stock in Subdivision 3Ps, and the northern Gulf of St. Lawrence stock in Divisions 3Pn4RS. A fifth stock, the Flemish Cap stock in Division 3M, lies outside of Canadian waters (Fig. 2.1). Prior to collapse, the Atlantic cod fishery was the largest fishery in Newfoundland and Labrador, with catches of about 240,000 tonnes annually during the 1980s from the northern cod stock (spanning from southern Labrador to the northern half of Grand Bank in NAFO Divisions 2J3KL) through the 1980s (Rose 2007; DFO 2016). Given the economic importance of the fishery and concerns about potential increases in anisakid nematode infection levels, Brattey and Bishop (1992) undertook an extensive study during 1985-1987 to determine the distribution and abundance of larval *A. simplex* s.l. in the flesh of cod from stocks around the province. Templeman et al. (1957) found abundance and density of *A. simplex* s.l. to be low in most areas around Newfoundland and Labrador during 1947-1953, with highest levels observed along the west and south coasts of Newfoundland. Brattey and Bishop (1992) found comparable infection levels

between studies in most areas during the 1980s, although the abundance of *A. simplex* s.l. appeared to have increased among cod along Newfoundland's south coast (the later investigators did not examine samples from the west coast of Newfoundland). The cause of this temporal variation in *A. simplex* s.l. abundance within Atlantic cod and apparent increase in some locations between these time periods is not well understood but has been hypothesized to relate to factors such as numbers of cetacean definitive hosts and cod diet (Templeman et al. 1957; Brattey and Bishop 1992).

With some cod stocks showing signs of improvement since the mid 2000s (Rose and Rowe 2015; DFO 2015b; DFO 2016), and ongoing fisheries in the southern Newfoundland stock in Subdivision 3Ps and the northern Gulf of St. Lawrence stock in Divisions 3Pn4RS, there has been renewed concern about anisakid nematode infection levels and highlights the importance of obtaining updated information on the occurrence of *A. simplex* s.l. The objective of the present study was to quantify infection levels of *A. simplex* s.l. in Atlantic cod stocks around Newfoundland and Labrador for comparison with data obtained during 1947-1953 (Templeman et al. 1957) and 1985-1987 (Brattey and Bishop 1992). Specifically, by examining Atlantic cod collected from separate stocks during 2012-2013, I evaluated: i) the location of larval *A. simplex* s.l. within the musculature of Atlantic cod; ii) whether prevalence, abundance and density of larval *A. simplex* s.l. within the musculature of Atlantic cod varied in relation to fish size or among stocks; and iii) whether there have been changes in the numbers of larval *A. simplex* s.l. within the musculature of Atlantic cod since the 1947-1953 and 1985-1987.

Terminology for nematode parasite infection statistics follows that of Margolis et al. (1982) who defined prevalence as the percentage of cod infected, abundance as the

mean number of *A. simplex* s.l. per cod including uninfected fish, density as the mean number of *A. simplex* s.l. per kilogram of flesh including uninfected fish, and mean intensity as the mean number of *A. simplex* s.l. per cod, including only infected individuals.

2.3 Materials and methods

To quantify prevalence, abundance and density of larval *A. simplex* s.l. in the musculature of Atlantic cod, samples of whole fish were collected in 2012 and 2013 using otter trawls during offshore surveys conducted by Fisheries and Oceans Canada, and the Centre for Fisheries Ecosystems Research of Memorial University. Stocks and areas sampled included the northern cod stock in NAFO Divisions 2J3KL, the southern Grand Bank stock in NAFO Divisions 3NO, the Flemish Cap stock in NAFO Division 3M, and the southern Newfoundland stock in Subdivision 3Ps (Fig. 2.1). In addition, samples were collected from the inshore sentinel research fishing activities conducted by local harvesters using long lines and gill nets; stock and areas sampled included the northern cod stock in Division 3L, the northern Gulf of St. Lawrence stock in Divisions 3Pn4R, and the southern Newfoundland stock in Subdivision 3Ps (Fig. 2.1). To the extent possible, samples were obtained from multiple sites within each stock area to document possible spatial heterogeneity in nematode infection levels. The size structure of cod samples I obtained differed among areas, but I attempted to sample as wide a size range as possible within each area to allow for comparisons of infection levels among length classes.

Upon capture, whole cod were labeled and frozen for examination in the laboratory. To facilitate comparison of my results with those of previous studies, I followed methods of Templeman et al. (1957) and Brattey and Bishop (1992). After thawing each fish, I measured the fork length (from the tip of the snout to the fork of the tail ± 1 cm). To quantify parasites in the musculature, flesh on the left and right sides of the fish was removed with a sharp knife, and fillets (dorsal musculature anteriorly and both dorsal and ventral musculature posteriorly) were separated from napes (hypaxial musculature that surrounds body cavity) by cutting where the ribs join the spinal cord and extending the cut posterioventrally to a point approximately at the middle of the anal fin. Left and right fillets and napes from each fish were skinned, weighed to the nearest gram, and examined for nematodes on a candling light table (Maritime Plastics, St. John's, NL). Musculature was sliced diagonally into sections approximately 3-5 mm thick to help reveal nematodes potentially deeply embedded in the flesh. Areas that were opaque due to any skin or membrane remaining on the musculature and areas with bruising or blood spots were investigated more thoroughly. Nematodes were removed using forceps and preserved in a solution of 1:9 glycerin to 70% ethyl alcohol (Brattey and Bishop 1992). Another parasitic nematode, *Pseudoterranova decipiens* s.l., occurs in the musculature of Atlantic cod (Brattey et al. 1990), but it differs distinctly in color, size, and morphology from *A. simplex* s.l. (Templeman et al. 1957). *Anisakis simplex* s.l. are small in size, and are white or lightly colored, whereas *P. decipiens* s.l. are much larger, and darkly colored. In cases of uncertainty, I identified specimens under a binocular dissecting microscope.

To determine my efficiency in detecting nematode larvae, I reinspected the flesh of 53 randomly selected cod that I had previously inspected using the candling and slicing

technique, using a pepsin-hydrochloric acid (HCl) digestion technique (Stern et al. 1958) that enables almost 100% of nematodes to be recovered (Brattey 1988; Brattey and Bishop 1992). Following inspection by candling and slicing, the combined musculature of each fish was placed in a 4 L beaker with a mixture composed of 1 L of warm water, 15 mL of 2N HCl (100%), and 2.5 g of pepsin, for each 250 g of flesh. Beaker contents were stirred occasionally to facilitate digestion and after approximately 20 hours filtered through a 400 µm sieve, rinsed with water, and examined in a clear glass tray over a black background with a short-wave ultraviolet (uv) light (this causes previously frozen nematodes to fluoresce, and aids in nematode detection). Nematodes recovered were extracted and preserved, as described above.

2.4 Statistical methods

The general pattern of infestation by *A. simplex* s.l. is presented as a summary of standard infection statistics in Table 2.1. For comparable summaries by earlier investigators, see Templeman et al. (1957), Brattey and Bishop (1992), Boily and Marcogliese (1995), and McClelland et al. (2011). Following these studies, I calculated infection statistics for each geographic area in 8 size groupings of cod (< 30 cm to ≥ 90 cm in 10-cm increments).

Standard statistical tests require samples to be independently and randomly selected, and so could not be used to analyze my data due to the clustered sampling technique (trawl or gillnet) used to collect fish; therefore the samples (‘sets’) were not random samples of the population. To account for clustering and possible heterogeneity of infection rates among sets within regions, the models to analyze both binomial

(infected vs. uninfected) and count data included fork length and stock as fixed-effects, and set as a random effect. Both models were fitted using the lmer algorithm from the lme4 package in R (Bates et al. 2013), which allows for an unbalanced design with both random and fixed-effects. The Akaike Information Criterion (AIC; Akaike 1973) was used for model selection. If the difference between AIC values between a model and the best model was less than 2, they could not be distinguished. Models with AIC that differed by 5 or more from the best model were considered to be improbable.

For most size groups, the variance in abundance was much greater than the mean, indicating that the distribution of parasite counts was not random in relation to cod size, but highly aggregated within a small proportion of cod harbouring high individual worm counts (i.e. the data are highly skewed). For example, the highest number of nematodes within one fish was 137 in a 60-cm fish caught on the Flemish Cap, therefore $\log(x+1)$-transformation of the counts was necessary. This method normalizes the residuals and fixes the skewness of the parasite distribution that occurs due to a small number of individuals that are highly infected (Wilson and Grenfell 1997).

A. simplex s.l. larvae recovered from the musculature were then grouped according to whether they were found in the left or right side of the nape, or fillet. G-tests of heterogeneity were used to test the null hypotheses that the number of nematodes did not differ between the right versus left side, or between nape and fillet. G-tests were performed using the RVAideMemoire package in R (Hervé 2015).

To test for an association between the number of *A. simplex* s.l. detected by candling and fork length, the Pearson product-moment correlation coefficient was computed.

Bar charts were used to illustrate infection rates between the surveys conducted during 1947-1953 (Templeman et al. 1957), 1985-1987 (Brattey and Bishop 1992) and the present. Samples from the present study were assigned as closely as possible into the regions defined in Figure 1 of Brattey and Bishop (1992) and Figure 11 of Templeman et al. (1957), and area names were given based on nearest landmarks (Figs. 2.1 and 2.2). Differences in cod size composition within regions between the current study and historical data sets precluded an analysis using all length classes, therefore, I only compared cod length ranges that were sufficiently sampled in both periods (all regions with $\geq$ 20 fish samples).

All data analyses and modeling were conducted using R 3.0.2 statistical package (R Core Team 2014) and significance was assessed at $\alpha = 0.05$.

2.5 Results

2.5.1 Summary of infection statistics by geographic region

A. simplex s.l. was common in the flesh of Atlantic cod in all regions sampled off Newfoundland and Labrador, and on the Flemish Cap (Table 2.1). The majority of length classes of cod from most areas had prevalence levels exceeding 60%. The only exceptions were cod with fork length < 30 cm (22%) and 30-39 cm (47%) in cod in NAFO Divisions 2J3KL, and < 30 cm (0%) in cod in Division 3NO.

The overall abundance of *A. simplex* s.l. in Atlantic cod in all regions around Newfoundland and Labrador ranged from 0.2 nematodes/fish (variance = 0.2) among fish < 30 cm in fork length in Divisions 2J3KL to 48.5 nematodes/fish (variance = 1379.8) among 60-69 cm in Division 3M (Table 2.1). Although abundance of *A. simplex* s.l. was

fairly low in NAFO Divisions 2J3KL (range 0.2 to 13.9 nematodes/fish) and cod in Divisions 3NO (range 0 to 8.1 nematodes/fish), infection levels were higher in samples (for most length classes) collected from Division 3Ps (range 1.4 to 11.2 nematodes/fish) and Divisions 3Pn4RS (range 1.4 to 27.0 nematodes/fish; Table 2.1). The 3M stock, located the furthest offshore and farthest east, had the highest infection levels (range 9.4 to 48.5 nematodes/fish; Table 2.1). In most areas there was a tendency for the abundance of *A. simplex* s.l. to increase with fish length (Table 2.1). For each stock, the number of *A. simplex* s.l. found in each length class of cod was highly variable (Table 2.1).

Mean density of larval *A. simplex* s.l. followed a similar pattern to prevalence and abundance, with the lowest densities (0 nematodes/kg) in NAFO Divisions 3NO in cod < 30 cm in fork length, and the highest densities (87.7 nematodes/kg, variance = 10 281) in Divison 3M in the 40-49 cm length class (Table 2.1). Divisions 3Pn4R also had high densities with 17.7 nematodes/kg overall. Even cod from the most lightly infected areas (Divisions 3Ps, 2J3KL, and 3NO) harboured substantial densities (> 4 nematodes/kg, excluding cod < 30 cm in fork length). In most areas, densities tended to increase with fish size, although they peaked in fish of intermediate size, typically between 40-59 cm (Table 2.1).

2.5.2 Statistical modelling of prevalence and abundance data

To further investigate the observed changes in the prevalence of *A. simplex* s.l. larvae, presence/absence data were analysed using a generalized linear mixed-effects model with a binomial error structure and logit link function. The model of best fit included *stock* and *length* as explanatory variables (AIC = 428; Table 2.2). However, the

fit was only marginally better than a model that included the interaction term (AIC = 433).

In this study, the variability in prevalence of *A. simplex* s.l. among trawl sets was accounted for by introducing a trawl-set specific random effect in the model. The random effect variance would approach zero if there was no variation in parasite numbers among sets and the data were taken from a homogeneous distribution or have high positive values if most parasites were found in a small proportion of the trawl sets. In my analysis the random effect variance was estimated at 0.07 (SD = ± 0.26), indicating that although variability among sets was low it was not zero and therefore, had to be accounted for in the model.

Table 2.3 provides the fixed-effect parameter estimates and standard errors from the generalized linear mixed-effects model [Equation 1] for prevalence data. These estimates are effectively the intercept terms on the logit scale. In this analysis the fixed estimates for each stock are tested to determine whether they differed significantly from zero on a logit scale which, when converted to a probability scale, is 0.5. Thus the significance values in Table 2.3 indicate the probability that the intercept term is significantly different from zero on a logit scale (= 0.5 or 50% prevalence on a probability scale). When they are converted to probabilities these intercept terms are the baseline prevalence of infection for a fish of 0 cm in length (i.e intercept on the y-axis). The values are much more meaningful on a probability scale (Table 2.3, values shown in brackets). For example, they show that the highest prevalence of infection at the intercept was found in the 3M stock (0.36) and the 3Pn4R stock (0.19). The results also show that only stocks 3M and 3Pn4R had non-significant intercept values ($p = 0.54$ and $p = 0.084$, respectively) relative

to a prevalence of 0.5. The β term in the model captures the extent to which prevalence increases in length within each stock [Equation 1].

An example is given below to illustrate how predicted prevalence is calculated using parameters from the modelled relationship between the stock estimate and fork length. In this example, I use a 50-cm fish from stock 3Pn4RS and parameter estimates are taken from Table 2.3. The equation is as follows:

$$y = e(\Theta_{S5} + (Y_L * \beta)) / (1 + e(\Theta_{S5} + (Y_L * \beta)) \quad [1]$$

$$y = e(-1.39 + (50 * 0.10)) / (1+ e(-1.39 + (50 * 0.10))$$

$$y = 0.974$$

Where, y = Probability of *A. simplex* s.l. infection

Θ_S = Stock effect estimate from the generalized linear mixed-effects model

S = Stock (1 = 2J3KL, 2 = 3NO, 3 = 3M, 4 = 3Ps, 5 = 3Pn4R)

Y_L = Length of fish in cm

β = Length effect estimate from the generalized linear mixed-effects model

Thus, the model [Equation 1] predicts a 97.3% probability of *A. simplex* s.l. infection for a cod 50-cm in fork length in stock 3Pn4RS. By comparison, the observed data show that cod in stock 3Pn4RS between 40-49 cm had a prevalence of 98.0%, and cod between 50-59 cm had a prevalence of 98.2% (Table 2.1).

The abundance data for *A. simplex* s.l. was analyzed using a linear mixed-effects

model following a log(x+1)-transformation. Stepwise model reduction was performed to identify the best fitting model (Table 2.4). The best fit was the linear mixed-effects model of log(x+1)-transformed counts of *A. simplex* s.l. larvae, which included *stock* and *length* as explanatory variables (AIC = 1845; Table 2.4). The fit was only marginally better than the model that included the covariate's interaction term (AIC = 1849).

Again, the variability in abundance of *A. simplex* s.l. between trawl sets was accounted for with a trawl-set specific random effect in the model. The random effect was estimated at 0.04 (SD = ± 0.21), indicating that although variability among sets was low it was not zero and therefore, had to be accounted for in the model.

A two-parameter model with set as a random effect fit the log(x+1)-transformed data on the abundance of *A. simplex* s.l. reasonably well for most stocks (e.g. 2J3KL, 3NO) but fit poorly for a few (e.g. 3Pn4R). The scatterplots of nematode abundance as a function of fish length illustrate the extremely high variability observed in the number of nematodes per fish within and among stocks (Fig. 2.3). A more complex model may be required to capture this type of extreme variability.

Table 2.5 provides the fixed-effect parameter estimates and standard errors from the linear mixed-effects model [Equation 2] for the abundance data. These estimates are on the log scale. When they are converted back to a linear scale (with one subtracted to account for the data transformation) they illustrate the baseline abundance for a fish of 0 cm in length (i.e intercept on the y-axis). Model estimates show that baseline abundance was highest in 3M (2.71) and 3Pn4R (0.92; Table 2.5). Standard errors for the fixed-effect estimates were slightly higher for the 3M and 3Pn4R stocks (0.20 and 0.16) which is reflected in the scatterplots of the relationship between length and number of nematodes

(Fig. 2.3) where there is a larger degree of variability in the number of nematodes within each length class for these two stocks (Fig. 2.3). The β term in the model captures the extent to which abundance increases with length within each stock [Equation 2].

A simple example is given below to illustrate how the model-predicted number of nematodes is calculated using the modelled relationship between the stock estimate and fish length. In this example, I use a 50-cm fish from stock 2J3KL, with parameter estimates taken from Table 2.5. The equation is as follows:

$$\text{Log}(x+1) = \Theta_{S1} + (\beta * Y_L) \qquad [2]$$

$$x = (e(-0.33 + (0.028 * 50)) -1$$

$$x = (e(-0.33 + 1.41)) -1$$

$$x = (e(1.07)) -1$$

$$x = 1.9$$

Where, x = Number of nematodes

Θ_S = Stock effect estimate from the linear mixed-effects model

S = Stock (1 = 2J3KL, 2 = 3NO, 3 = 3M, 4 = 3Ps, 5 = 3Pn4R)

β = Length effect estimate from the linear mixed-effects model

Y_L = Length of fish in cm

Thus, the model [Equation 2] predicts 1.9 nematodes/fish for a 50-cm long cod in stock 2J3KL. This is well within the range of observed values for a 50-cm fish from 2J3KL (Fig. 2.3). The observed values and model predicted estimates of log(x+1)-

transformed data for each stock are illustrated in Fig. 2.3. These scatter plots indicate a wide range of infection among individual fish for all areas. Most of the observed values are for cod that lie within the length range of 50-70 cm, as this reflects the most common length of cod investigated in most of the stocks.

2.5.3 Distribution within the musculature of Atlantic cod

The distribution patterns of *A. simplex* s.l. larvae between left and right fillets, and napes were generally similar over a wide range of cod sizes (Table 2.6). In all size groups the napes harboured most nematodes (range 97% to 98%), while the fillets harboured only a small percentage (< 3%).

Within samples from all size groups there were more *A. simplex* s.l. in the left side of the flesh for both fillet (total 53%) and nape (total 61%) samples (Table 2.6). For the pooled data (when nematode counts from all stocks and fish sizes were combined), the difference between left and right sides was significant for napes (G = 277, DF = 1, $p < 0.001$), but not for fillets (G = 0.42, DF = 1, $p = 0.52$). The total G value was also significant for the nape (G = 287, DF = 4, $p < 0.001$) but not for fillets (G = 1.24, DF = 4, $p = 0.87$). There was significant heterogeneity among size groups between the numbers of *A. simplex* s.l. in left versus right sides for napes, but not for fillets (Table 2.6). This indicates that the distribution of *A. simplex* s.l. in the left and right sides of the nape, but not the fillets, differed significantly among size groups. Although more than half of the nematodes were found in the left nape for all size groups, some had much more than half, which yielded larger G values for the individual length classes (fork length 24-117 cm).

Sample sizes (numbers of *A. simplex* s.l.) were much smaller for fillet portions, so the statistical power of the *G*-tests for each size group was lower than for the napes.

2.5.4 Detection rate

The musculature of 53 randomly selected cod (mean fork length ± SE = 56.7 ± 1.7 cm, range 36-106 cm) collected in NAFO Divisions 4R and 3M were re-inspected for *A. simplex* s.l. larvae using a pepsin-HCl digestion which recovers nematodes missed during candling and slicing. This procedure indicated that percentage recovery for *A. simplex* s.l. during candling and slicing was 80.5% (Table 2.7). By comparison, Brattey and Bishop (1992) reported only 42.1% recovery, thus necessitating that a standardized factor be applied to ensure measures of parasite abundance can be comparable between studies and results are not a product of respective recovery rates. Although Templeman et al. (1957) did not report detection rate of nematodes as part of their investigation, it is assumed to be similar to that documented Brattey and Bishop (1992) given that similar methods were employed and some individuals participated in both studies.

No association existed between nematode detection rate and fish length ($r = -0.09$, $DF = 51$, $p = 0.51$).

2.5.5 Comparison with previous surveys

I compared my results with two earlier studies. A formal statistical modelling analysis of temporal changes in parasite numbers was not possible because the raw data from Templeman et al. (1957) were not available and variance was not reported; therefore my results could only be compared with those in published summary tables. In addition,

Templeman et al. (1957) did not report their detection efficiency, and the detection efficiency reported by Brattey and Bishop (1992) was considerably different from the current study, so nematode counts from individual fish could not be standardized to facilitate comparison across studies.

To investigate whether infection levels had changed over time, I first compared the prevalence, abundance and mean density between my data and those of Brattey and Bishop (1992; Table 2.8; Fig. 2.1). There were insufficient samples from some regions for comparison, so only regions with ≥ 20 samples were included. Brattey and Bishop (1992) presented summary information on the abundance and mean density of cod within groups of 10-cm increments. The present data were similarly grouped for comparison to the results of Brattey and Bishop (1992). Based on these results, all size groups within all regions, with the exception of cod 40-49 cm from southern Funk Island Bank, had higher abundances of *A. simplex* s.l. in 2012-2013 than in 1985-1987 (Table 2.8). Although some size groups had small sample sizes for comparison, the data indicate that abundance in all areas has increased from 1985-1987 to 2012-2013, though the most recent study also had higher variance.

Since the detection rates were drastically different between the two studies, the overall abundance for each size group was calculated following a transformation based on standardized factors (= mean abundance * (1/detection rate)). The standardized abundances remained higher for all regions in 2012-2013 (with the exception of cod 40-49 cm from southern Funk Island Bank). Variance could not be standardized and therefore caution must be used when directly comparing the infection levels using this method.

Bar charts for standardized abundance and standardized mean density were created using the lattice package (Sarkar 2008) in R statistical software to compare infection levels of *A. simplex* s.l. between 1985-1987 (Brattey and Bishop 1992) and 2012-2013 (Fig. 2.4 and 2.5). While there was higher variability in the current study based on the raw abundance, the standardized abundance and density were also generally greater in the current study than the 1980s in all regions, indicating an increase in infection levels.

I also compared three time periods, 1947-1953 (Templeman et al. 1957), 1985-1987 (Brattey and Bishop 1992), and the samples collected in 2012-2013 (Table 2.9). Data in Table IV of Templeman et al. (1957) were presented as the number of nematodes and the proportion of *A. simplex* s.l. in the fillets of cod ≥ 41 cm (only fillets were used in this study, napes were excluded). Using this information, Brattey and Bishop (1992) derived the abundance and the density of *A. simplex* s.l. in the fillets from their data. The same measures from the current data were calculated and the results were compared across nine areas (Fig. 2.2). The values presented for 1957 were taken from Figure 11 and Table IV in Templeman et al. (1957) (Fig. 2.2; Appendices C and D). Brattey and Bishop (1992) did not survey the Flemish Cap or the west coast of Newfoundland, so the results from the current study only compare abundance and density to Templeman et al.'s (1957) results for those regions. As Templeman et al. (1957) did not provide estimates of variance around the mean and raw data were not available, statistical analyses of infection levels were not possible. The data were compared after standardization factors were applied to the mean. Since Templeman et al. (1957) recorded only *A. simplex* s.l. larvae found in the fillets of cod and most nematodes of this species are found in the napes, the

results presented may not be a representation of infection levels, but rather the changes in the number of nematodes occurring in the fillets between studies.

In areas that were compared to the present study, increases were seen in the standardized abundance and densities in the fillets from 1947-1953 to 1985-1987 in all regions (Table 2.9). In the current study there was a substantial increase in infection levels compared to historical data. The only exception where standardized abundance was higher in 1985-1987 compared to present levels was on the south coast of Newfoundland, from Pass Island to Cape Ray, although standardized density was higher in the present study. Otherwise, standardized abundance and density had increased more than two-fold or greater in some areas (Table 2.9). Bar charts were created using standardized abundance to illustrate differences in infection levels of *A. simplex* s.l. among the three surveys in 1947-1953 (Templeman et al. 1957), 1985-1987 (Brattey and Bishop 1992), and 2012-2013 (Fig. 2.6). In general, an increasing trend in infection levels was observed with a much steeper increase from 1985-1987 to 2012-2013 in most areas surveyed.

2.6 Discussion

This study revealed geographic variation in the intensity of *A. simplex* s.l. in the musculature of Atlantic cod off Newfoundland and Labrador, as well as variation among length classes. *Anisakis simplex* s.l. continues to be a widely distributed nematode in the Northwest Atlantic and commonly found in Atlantic cod off Newfoundland and Labrador as well as the Flemish Cap. The data suggests an overall increase in abundance and density since the surveys of Brattey and Bishop (1992) and Templeman et al. (1957). Across NAFO divisions, I found a geographic trend of increasing infection levels towards

more easterly areas, with the highest levels on the Flemish Cap. A deviation from the trend was NAFO 3Pn4R stock, the most westerly area, which had the second highest infection levels. Although the viscera of cod were not examined for *A. simplex* s.l. larvae in the present study, this is where most reside (McClelland et al. 1990; Brattey and Bishop 1992) and therfore the overall abundance of this nematode is much greater than presented.

Anisakis simplex s.l. is a complex of species, with *A. simplex* s.str. being the primary species infecting marine fishes around Newfoundland and Labrador (Brattey and Bishop 1992; Mattiucci et al. 1997) and therefore most likely to be the species observed in this study.

Boily and Marcogliese (1995) surveyed *A. simplex* s.l. in the flesh of Atlantic cod from multiple areas in the Gulf of St. Lawrence, which included off southwestern Newfoundland (Subdivision 4R), an area also sampled in the current study. Based on Table 1 in Boily and Marcogliese (1995), the overall abundance of *A. simplex* s.l. in the flesh of cod from this area was 0.45 nematodes/fish (SE = ± 0.12), with an overall prevalence of 20%. I sampled this areaa in my study, and found a prevalence of 97% and 11.9 nematodes per fish, suggesting an increase in parasite population since 1992.

My study, and that of Brattey and Bishop (1992) point to the need for caution when interpreting and comparing results from different parasite surveys. Detection of larval parasites (*A. simplex* s.l. in particular) varies greatly across observers. As noted, Brattey and Bishop (1992) detected nematodes in the flesh of cod with 42% efficiency, vs. 81% in my study. Despite the large difference in detection rate between studies, the overall conclusions were similar after standardizing infection values by detection rates. Future

surveys should quantify and report detection efficiency to ensure that results are not biased by variable detection rates and can be compared between surveys.

Differences in sample sizes and length groupings may also influence the results. Sample sizes in my study were much smaller than previous studies, particularly for the larger length classes: some stocks off Newfoundland and Labrador have not recovered since the collapse of groundfish in the late 1980s and early 1990s, making it more difficult to attain samples of large fish. However, I addressed differences in size structure over time and among populations by controlling for fish size in many analyses.

Information on population trends for cetaceans (the main definitive host of *A. simplex* s.l.) that migrate, forage, or reside around Newfoundland and Labrador is limited, making it difficult to assess which species are most important in the transmission and distribution of the parasite. Some whale and dolphin populations may be increasing (Stevick et al. 2003; Benjamins et al. 2012; Magera et al. 2013), but population trends for most species are not well known. Moreover, only small numbers of some cetacean species have been examined for adult *A. simplex* s.l., mostly through incidental by-catch and strandings (Brattey and Stenson 1995; Hays et al. 1998b; Ugland et al. 2004). Therefore infection levels are not known for most species. Without better information on trends in cetacean population size, as well as their level of infection with reproductive adults of *A. simplex* s.l., it is impossible to determine if the higher infection levels of larval *A. simplex* s.l. in cod are correlated with population changes among cetacean definitive hosts. Furthermore, the information in the literature regarding the life cycle of *A. simplex* s.l. is limited, making it difficult to understand which species of cetaceans harbour the highest intensities of *A. simplex* s.l. Additional research, such as the occurrence of parasites in

cetaceans, would be valuable and would provide insight as to why increases in abundance have been observed since previous parasitological surveys were conducted.

Other factors may also be contributing to the increasing infection levels observed in this study. Warming trends in the Atlantic Ocean accelerate the hatching times, development and survival of *A. simplex* s.l. (Brattey and Clark 1992; McClelland et al. 2011). Increasing bottom temperatures have also been recorded in northern areas and on the Flemish Cap (Colbourne et al. 2014). The warming trend seen over the past few years in the North Atlantic (Colbourne et al. 2014) may partly explain the increased levels of *A. simplex* s.l. infection in the flesh of cod. Should this warming trend continue, it is likely that there will continue to be an increase in the abundance and prevalence of larval anisakine nematodes found in not only Atlantic cod, but any species that may become host to these parasites.

My results show that prevalence and abundance tend to increase with cod length, a finding that is consistent with other studies of larval *A. simplex* s.l. both in cod and other fish hosts (Brattey and Bishop 1992; Marcogliese 1995). Increasing variability in abundance with host length is also commonly observed in these field studies. Cod acquire larval stages of *A. simplex* s.l. from prey and some invertebrate euphausiid prey species, such as northern krill (*Meganyctiphanes norvegica*) and Arctic krill (*Thysanoessa raschii*), have been identified as important intermediate hosts (Hays et al. 1998a). Infection levels in euphausiids are typically extremely low, with only a few individual larvae of *A. simplex* s.l. per thousand euphausiids, and smaller cod that feed mostly on macroinvertebrates likely acquire these nematodes individually (Hays et al. 1998a). However, as cod grow, the volume of food that is ingested increases and they shift to a

more piscivorous diet that includes fish species known to harbour multiple infections (Marcogliese 1995; Link and Garrison 2002; Sherwood et al. 2007). Consequently, larger and older cod ingest much greater numbers of larval nematodes with more heavily infected prey. Given that larval *A. simplex* s.l. is described as long lived and accumulates in fish over time (Smith 1984; Klimpel et al. 2004), increases in prevalence and abundance with length are expected and are a common feature of larval *A. simplex* s.l. and other ascaridoid nematode infections in fish (Brattey et al. 1990; Brattey and Bishop 1992; Boily and Marcogliese 1995; Marcogliese 1995; Martell and McClelland 1995).

Specific mechanisms for the generally higher infection levels of cod with *A. simplex* s.l. in my study compared with previous surveys are difficult to identify. This nematode is transmitted to cod up the food chain through multiple pathways involving macroinvertebrates such as euphausiids and many fish species during predator-prey interactions. With significant changes in the structure of fish and invertebrate communities in Northwest Atlantic ecosystems, this would also generate changes in the diets of many species. An increase in certain fishes, such as Atlantic herring and American plaice, has been observed in the diet of Atlantic cod in the southern Gulf of St. Lawrence and on the eastern Scotian Shelf (Hanson and Chouinard 2002; Bundy and Fanning 2005). Atlantic herring has been known to harbour substantial abundances of *A. simplex* s.l. (Hays et al. 1998b). This shift in diet, particularly towards prey species that are preferred hosts of *A. simplex* s.l., could have generated the increases observed in my study.

The results support previous findings that more *A. simplex* s.l. larvae migrate into the napes of cod, particularly the left side (Brattey and Bishop 1992). Possible explanations

have been suggested based on the asymmetric organization of the viscera of cod (McClelland et al. 1985; Smith and Hemmingsen 2003). The left liver lobe dominates the body cavity and is continuous with the stomach wall, so nematodes may encounter the left liver lobe and follow the easiest route to the musculature from there, or they may be hepatotropic (McClelland et al. 1985; Smith and Hemmingsen 2003).

Many studies have also found that *A. simplex* s.l. larvae less often migrate deeply into the fillet (McClelland et al. 1990; Brattey and Bishop 1992) and a similar trend was observed in the current study. The majority of the larvae found in the flesh reside in the nape, and removal of the napes can limit the number of nematodes by greater than 90%. When inspecting the napes for larvae, the left side should be inspected more thoroughly as there are more nematodes that migrate to this side. Although the viscera of cod were not examined for *A. simplex* s.l. larvae in the present study, this is where most reside (McClelland et al. 1990; Brattey and Bishop 1992).

In terms of food processing, removing the napes from the rest of the fillet would greatly reduce the percentage of the nematodes found within the remaining flesh. It is also important to advise fishermen not to dump viscera or napes back into the sea after gutting fish as this could potentially allow the nematodes to reenter a new host and perpetuate the increasing numbers of nematodes in marine organisms (McClelland et al. 1990).

Candling is the current technique utilized for removal of nematodes from the musculature of fish. In addition to being extremely labour-intensive, this method can miss many parasites that are encysted > 6 mm deep in the musculature (Bublitz and Choudhury 1992), where they are difficult to detect. Furthermore, detection efficiencies vary across observers; detection of encysted nematodes may vary between 33-93 % (McClelland

2002). Efforts have been made to develop and implement novel methods of detection and removal of parasites, such as imaging spectroscopy (Heia et al. 2007), electromagnetic detection (Choudhury and Bublitz 1994), and sensitive magnetometer detection (Jenks et al. 1996), but candling remains the only practical option at this time (McClelland 2002).

Overall there have been substantial increases in the infection of *A. simplex* s.l. L3 larvae in the flesh of Atlantic cod in most length classes and all areas surveyed around Newfoundland and Labrador, and on the Flemish Cap, since previous studies were conducted. Reasons for the increase are unclear, but may relate to overall ecological changes in the Northwest Atlantic ecosystem. It would be expected that parasites such as *A. simplex* s.l., which are transmitted through predator-prey interactions, have been affected by these changes (Marcogliese 2001; 2002).

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Table 2.1 Infection statistics for *Anisakis simplex* s.l. in the musculature of Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and the Flemish Cap during 2012-2013. Prevalence: percentage of fish infected; Abundance: mean number of nematodes per fish including uninfected fish (nematodes/fish); Maximal abundance: maximal number of nematodes in a single fish; Mean density: mean number of nematodes per kilogram of musculature (skinned nape and fillet) including uninfected fish (nematodes/kg); Mean Intensity: mean number of nematodes in infected fish excluding uninfected fish (nematodes/infected fish).

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
2J3KL	< 30	9	22.2 (6.3-54.7)	0.2 (0.2)	1	4.6	1.0
2012: July, November,	30-39	15	46.7 (24.8-69.9)	0.8 (1.2)	3	4.8	1.7
December; 2013: May	40-49	35	60.0 (43.6-74.4)	2.5 (18.4)	17	7.8	4.1
	50-59	27	96.3 (81.7-99.8)	4.2 (8.4)	14	7.2	4.3

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
	60-69	30	93.3 (78.7-98.2)	5.7 (20.7)	23	6.1	6.1
	70-79	17	94.1 (73.0-99.7)	5.9 (32.4)	21	4.7	6.2
	80-89	23	95.7 (79.0-99.8)	6.1 (13.8)	14	3.0	6.4
	≥ 90	6	100.0 (61.0-100.0)	13.8 (205.0)	35	4.4	13.8
	Total	162	79.0 (72.1-84.6)	4.4 (27.9)	35	4.9	5.5
3M	30-39	24	95.8 (79.8-99.8)	9.4 (101.1)	42	74.8	9.8
2012: May-June; 2013:	40-49	22	95.5 (78.2-99.8)	24.0 (752.7)	100	87.7	25.1
April	50-59	15	100.0 (79.6-100.0)	24.9 (209.5)	52	43.1	24.9
	60-69	13	100.0 (77.2-100.0)	48.5 (1379.8)	137	57.0	48.5
	70-79	10	100.0 (72.2-100.0)	33.1 (669.2)	105	26.6	33.1
	80-89	14	100.0 (78.5-100.0)	26.2 (160.3)	51	14.5	26.2

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
	≥ 90	7	100.0 (64.6-100.0)	20.7 (88.2)	39	7.4	20.7
	Total	105	98.1 (93.3-99.5)	24.8 (578.0)	137	30.3	25.3
3NO	< 30	5	0 (NA)	0.0 (NA)	0	0	NA
2012: September-	30-39	46	43.5 (30.2-57.8)	0.8 (1.3)	4	4.9	1.9
October	40-49	42	69.0 (54.0-80.9)	1.8 (4.9)	10	5.4	2.6
	50-59	44	81.8 (68.0-90.5)	4.2 (32.5)	33	7.3	5.1
	60-69	27	92.6 (76.6-97.9)	6.1 (63.3)	41	6.5	6.6
	70-79	9	88.9 (56.5-99.4)	8.1 (31.6)	18	5.2	9.1
	80-89	3	100.0 (43.9-100.0)	6.3 (10.3)	10	2.6	6.3
	≥ 90	7	100.0 (64.6-100.0)	4.0 (5.3)	7	0.7	4.0
	Total	183	69.9 (62.9-76.1)	3.2 (24.9)	41	4.4	4.5

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
3Ps	30-39	13	76.9 (49.7-91.8)	1.4 (1.1)	3	10.0	1.8
2012: April-May;	40-49	37	94.6 (82.3-98.5)	4.6 (25.2)	21	16.2	4.9
2013: May	50-59	82	95.1 (88.1-98.1)	5.4 (26.3)	25	10.7	5.6
	60-69	49	100.0 (92.7-100.0)	7.4 (28.0)	27	10.0	7.4
	70-79	21	100.0 (84.5-100.0)	7.5 (22.4)	17	6.1	7.5
	80-89	5	100.0 (56.6-100.0)	11.2 (30.2)	19	4.9	11.2
	Total	207	95.7 (91.9-97.7)	5.8 (27.2)	27	9.5	6.1
3Pn4R	30-39	7	71.4 (35.9-91.8)	1.4 (2.3)	4	6.9	2.0
2012: October; 2013:	40-49	51	98.0 (89.7-99.9)	7.1 (69.1)	37	20.9	7.3
July-September	50-59	56	98.2 (90.6-99.9)	13.1 (153.1)	57	21.6	13.3
	60-69	38	100.0 (90.8-100.0)	12.9 (83.9)	36	13.5	12.9

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
	70-79	1	100.0 (5.1-100.0)	4.0 (NA)	4	3.3	4.0
	80-89	1	100.0 (5.1-100.0)	27.0 (NA)	27	15.5	27.0
	Total	154	97.4 (93.5-99.0)	10.6 (111.5)	57	17.7	10.8

* Density was calculated for each individual fish (nematodes/kg), then the mean of those densities was calculated within each length class for the Mean Density.

Table 2.2 Comparison of model fits for the binomial data on infection (infected = 1, not infected = 0) for Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and the Flemish Cap infected with *Anisakis simplex* s.l. using a logit link function and binomial error structure. The asterisk (*) indicates an interaction term is included as a parameter in the model. The model of best fit is indicated in bold.

R model					
Fixed-effects	Random effects	DF	AIC	Log likelihood	Deviance
Infected ~ stock + length	**+ (1|set)**	**7**	**428**	**-207**	**414**
Infected ~ stock * length	+ (1|set)	11	433	-205	411
Infected ~ 0 + length	+ (1|set)	2	488	-242	484
Infected ~ 0 + stock	+ (1|set)	6	523	-256	511
Infected ~ 0	+ (1|set)	1	596	-297	594

Table 2.3 Parameter estimates for a generalized linear mixed-effects model [equation 1] with binomial error structure and logit link function fitted to binomial (prevalence) data of *Anisakis simplex* s.l. in Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and the Flemish Cap. Values in parentheses are stock estimates transformed to probability of infection (i.e., prevalence: percentage of fish infected).

Fixed-effects	Stock effect estimate	Standard error	z-value	p
Length (β)	0.10	0.01	7.56	< 0.0001
2J3KL (Θ_{S1})	-3.85 (0.02)	0.66	-5.83	< 0.0001
3M (Θ_{S2})	-0.57 (0.36)	0.92	-0.62	0.5360
3NO (Θ_{S3})	-3.91 (0.02)	0.64	-6.09	< 0.0001
3Ps (Θ_{S4})	-2.07 (0.11)	0.71	-2.93	0.0034
3Pn4R (Θ_{S5})	-1.39 (0.19)	0.80	-1.73	0.0842

Table 2.4 Comparison of AIC values to determine best fitting linear mixed-effects model for log(*x*+1)-transformed counts of *Anisakis simplex* s.l. in Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and the Flemish Cap during 2012-2013. The asterisk (*) indicates an interaction term is included as a parameter in the model. The model of best fit is indicated in bold.

R model					
Fixed-effects	Random effects	DF	AIC	Log likelihood	Deviance
Log(*x*+1) ~ stock + length	**+ (1|set)**	**8**	**1845**	**-914**	**1829**
Log(*x*+1) ~ stock * length	+ (1|set)	12	1849	-912	1825
Log(*x*+1) ~ 0 + length	+ (1|set)	3	1890	-942	1884
Log(*x*+1) ~ 0 + stock	+ (1|set)	7	2021	-1003	2007
Log(*x*+1) ~ 0	+ (1|set)	2	2154	-1075	2150

Table 2.5 Parameter estimates for a linear mixed-effects model [Equation 2] of log(x+1)-transformed count data of *Anisakis simplex* s.l. in Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and the Flemish Cap. Values in parentheses are stock estimates back-transformed to a linear scale.

Fixed-effects	Stock effect estimate	Standard error	T-value
Length (β)	0.03	< 0.01	14.36
2J3KL (Θ_{S1})	-0.33 (-0.28)	0.01	-2.43
3M (Θ_{S2})	1.31 (2.71)	0.20	6.66
3NO (Θ_{S3})	-0.41 (-0.34)	0.12	-3.35
3Ps (Θ_{S4})	0.04 (0.04)	0.15	0.29
3Pn4R (Θ_{S5})	0.65 (0.92)	0.16	3.99

Table 2.6 Distribution of larval *Anisakis simplex* s.l. in the musculature (left and right nape and fillet) of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador and on the Flemish Cap during 2012-2013. The null hypothesis being tested was that nematodes are equally distributed between left and right sides of nape and fillet portions for four length classes of cod.

		Nape				Fillet				
Length class (cm)	No. of cod	No. of *A. simplex*	% in left side	G	P	No. of *A. simplex*	% in left side	G	P	% of total *A. simplex* in napes
40-49	187	1204	63.7	91.6	***	21	47.6	0.05	n.s	98.3
50-59	224	1796	62.7	117.0	***	48	54.2	0.33	n.s	97.4
60-69	157	1782	58.7	54.2	***	37	51.4	0.03	n.s	98.0
70-79	58	654	59.6	24.4	***	11	63.6	0.83	n.s	98.3
Pooled	626	5436	61.2	277.1	***	117	53.0	0.42	n.s	
		Total		287.3	***	Total		1.24	n.s	97.9
		Heterogeneity		10.24	*	Heterogeneity		0.82	n.s	

Signifiance: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' > 0.1 'n.s '
Degrees of Freedom: Individual length classes = 1, pooled = 1, Total = 4, Heterogeneity = 3

Table 2.7 Percentage of *Anisakis simplex* s.l. recovered from the musculature of Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and the Flemish Cap during 2012-2013 using the candling and slicing technique. Total: number of nematodes recovered candling in addition to the number of nematodes found using subsequent HCL digestion.

Length class (cm)	No. of fish	Mean fork length (cm)	Prevalence (%)	Abundance ± SE	Maximal abundance	Number recovered candling	Total	Recovery rate (%)
35-44	6	41.3	100.0	3.8 ± 0.8	6	21	23	91.3
45-54	18	49.1	100.0	16.7 ± 3.7	63	238	300	79.3
55-64	19	59.4	100.0	19.0 ± 3.3	51	297	362	82.0
65-74	7	67.3	100.0	16.9 ± 5.2	39	86	118	72.9
≥ 75	3	90.7	100.0	25.0 ± 7.4	32	65	75	86.7
Total	53	56.7	100.0	16.6 ± 1.9	63	707	878	80.5

Table 2.8 Sample sizes, mean abundance (variance), standardized mean abundance and standardized mean density (standardized numbers were calculated by multiplying the mean abundance or density by 1/detection rate, based on respective detection rates) of *Anisakis simplex* s.l. in the musculature of Atlantic cod (*Gadus morhua*) sampled from various areas surrounding Newfoundland and Labrador, and the Flemish Cap during 1985-1987 (Brattey and Bishop 1992) and 2012-2013. Sampling areas identified below correspond to those in Figure 1 of Brattey and Bishop (1992; Appendix B) and reference numbers correspond to Fig. 2.1.

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
Southern	30-39	0	9	NA	0.8 (1.2)	NA	0.97	NA	5.7
Funk Island	40-49	172	2	0.28 (0.4)	0.0 (0.0)	0.67	0.0	1.6	0.0
Bank (1)	50-59	758	1	0.57 (1.2)	3.0 (NA)	1.35	3.7	2.2	6.4
	60-69	335	7	0.90 (1.7)	5.9 (8.1)	2.14	7.3	2.3	8.0

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	70-79	81	8	0.86 (1.4)	3.4 (12.8)	2.04	4.2	1.5	3.9
	80-89	21	12	0.71 (1.1)	6.8 (17.6)	1.69	8.5	0.8	4.4
	≥ 90	0	3	.	5.3 (6.3)	NA	6.6	NA	2.2
	Total	1367	42	0.63 (1.3)	4.2 (14.8)	1.50	5.2	2.1	4.4
Northeast	< 30	0	9	NA (NA)	0.2 (0.2)	NA	0.3	NA	5.7
Newfoundlan	30-39	13	0	0.00 (0.0)	NA (NA)	0.00	NA	0.0	NA
d Shelf (2)	40-49	246	1	0.26 (0.4)	1.0 (NA)	0.62	1.2	1.4	1.5
	50-59	851	9	0.73 (1.2)	5.1 (16.4)	1.73	6.4	2.8	10.8
	60-69	406	7	1.03 (1.9)	3.6 (10.3)	2.45	4.4	2.6	5.0
	70-79	95	1	1.23 (2.4)	21.0 (NA)	2.92	26.1	2.1	16.2
	80-89	17	4	0.65 (1.0)	5.0 (15.3)	1.54	6.2	0.7	2.8

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	≥ 90	17	1	0.41 (0.6)	29.0 (NA)	0.97	36.0	0.3	10.8
	Total	1645	32	0.75 (1.4)	4.5 (41.9)	1.78	5.6	2.3	6.8
Grand Bank (3)	30-39	29	6	0.03 (0.0)	0.8 (1.4)	0.07	1.0	0.5	6.5
	40-49	70	9	0.10 (0.1)	6.7 (46.7)	0.24	8.3	0.7	26.0
	50-59	178	8	0.66 (1.4)	4.4 (4.0)	1.57	5.4	2.8	9.2
	60-69	122	8	1.17 (2.2)	5.9 (9.5)	2.78	7.3	3.1	8.0
	70-79	86	3	0.92 (1.6)	7.0 (49.0)	2.19	8.7	1.6	6.4
	80-89	32	2	0.59 (0.5)	4.0 (8.0)	1.40	5.0	0.7	2.6
	≥ 90	35	1	0.43 (0.5)	35.0 (NA)	1.02	43.5	0.3	12.3
	Total	552	37	0.69 (1.4)	5.7 (44.9)	1.64	7.1	1.7	9.6
	< 30	0	1	NA (NA)	0.0 (NA)	NA	0.0	NA	0.0

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	30-39	19	30	0.05 (0.0)	0.50 (0.9)	0.12	0.6	0.9	3.6
	40-49	20	24	0.05 (0.4)	2.0 (6.9)	0.12	2.5	0.4	7.1
Southeast	50-59	22	33	0.41 (0.2)	3.3 (14.9)	0.97	4.1	1.9	7.1
Shoal of the	60-69	20	20	0.25 (0.7)	5.9 (80.7)	0.59	7.3	0.7	7.3
Grand Bank	70-79	27	7	0.41 (0.4)	7.1 (30.5)	0.97	8.9	0.7	5.7
(4)	80-89	27	3	0.26 (0.8)	6.3 (10.3)	0.62	7.9	0.3	3.2
	≥ 90	115	7	0.50 (0.5)	4.0 (5.3)	1.19	5.0	0.3	0.9
	Total	250	125	0.36 (NA)	3.1 (24.0)	0.86	3.8	0.3	4.4
Whale Bank	< 30	0	4	NA (NA)	0.0 (0.0)	NA	0.0	NA	0.0
(5)	30-39	31	16	0.10 (0.1)	1.4 (1.7)	0.24	1.8	2.0	10.8
	40-49	29	18	0.14 (0.2)	1.6 (2.4)	0.33	1.9	1.3	6.1

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	50-59	55	11	0.18 (1.0)	6.9 (80.9)	0.43	8.6	0.9	15.1
	60-69	63	6	0.67 (1.7)	5.8 (15.4)	1.59	7.2	2.0	9.1
	70-79	36	2	0.83 (1.0)	11.5 (40.5)	1.97	14.3	1.6	9.0
	80-89	27	0	0.96 (1.0)	NA (NA)	2.28	NA	1.3	NA
	≥ 90	102	0	0.88 (1.2)	NA (NA)	2.09	NA	0.5	NA
	Total	343	57	0.60 (NA)	3.2 (26.1)	1.43	4.0	0.8	10.1
Placentia Bay	30-39	22	1	0.05 (0.05)	3.0 (NA)	0.12	3.7	0.7	19.9
(9)	40-49	57	13	0.16 (0.2)	7.9 (47.4)	0.38	9.8	1.2	29.3
	50-59	56	25	0.57 (0.9)	8.3 (38.3)	1.35	10.3	2.4	18.1
	60-69	57	2	1.04 (2.3)	16.5 (220.5)	2.47	20.5	2.7	25.6

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	70-79	49	1	1.14 (1.5)	5.0 (NA)	2.71	6.2	2.1	3.2
	80-89	19	1	1.21 (2.6)	11.00 (NA)	2.87	13.7	1.5	5.6
	≥ 90	13	0	0.31 (0.4)	NA (NA)	0.74	NA	0.2	NA
	Total	273	43	0.67 (1.3)	8.4 (45.0)	1.59	10.4	1.7	18.1
Green Bank	30-39	0	6	NA (NA)	1.3 (0.7)	NA	1.7	NA	12.2
(6)	40-49	38	15	0.29 (0.4)	3.3 (6.3)	0.69	4.1	1.7	14.6
	50-59	250	51	0.52 (0.8)	4.2 (17.5)	1.24	5.2	2.1	10.9
	60-69	189	47	0.96 (1.8)	7.0 (20.6)	2.28	8.7	2.6	11.9
	70-79	50	15	1.62 (2.9)	8.0 (18.0)	3.85	9.9	2.7	8.4
	80-89	29	3	0.72 (1.1)	8.7 (20.3)	1.71	10.8	0.8	4.5
	≥ 90	29	0	1.14 (2.0)	NA (NA)	2.71	NA	0.8	NA

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	Total	585	137	0.78 (1.5)	5.4 (19.9)	1.85	6.8	2.0	10.5
Southern St.	30-39	0	6	NA (NA)	1.2 (1.4)	NA	1.5	NA	11.0
Pierre Bank	40-49	15	9	0.27 (0.2)	2.2 (3.4)	0.64	2.8	1.6	11.9
(7)	50-59	171	6	0.66 (1.0)	3.5 (5.9)	1.57	4.4	2.5	9.3
	60-69	145	1	1.22 (2.1)	12.0 (NA)	2.90	14.9	3.1	17.1
	70-79	59	5	1.98 (3.0)	6.4 (44.8)	4.70	8.0	3.2	6.3
	80-89	9	1	0.78 (0.9)	19.0 (NA)	1.85	23.6	0.8	12.8
	Total	399	28	1.05 (1.9)	4.0 (23.7)	2.49	4.9	2.8	9.4
Rose Blanche	30-39	18	7	0.44 (1.6)	1.4 (2.3)	1.05	1.8	8.8	8.6
Bank (11)	40-49	155	51	1.15 (2.3)	7.1 (69.1)	2.73	8.8	7.9	26.0

Sampling Area (Reference number)	Length class (cm)	No. of fish		Mean Abundance (var)		Standardized Mean Abundance		Standardized Mean Density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	50-59	317	28	1.71 (3.4)	12.4 (147.5)	4.06	15.4	7.7	31.6
	60-69	94	6	2.83 (6.0)	10.8 (164.6)	6.72	13.4	8.3	14.0
	70-79	25	1	3.00 (8.3)	4.0 (NA)	7.13	5.0	5.5	4.0
	80-89	19	1	1.79 (2.6)	27.0 (NA)	4.25	33.5	2.1	19.3
	≥ 90	32	0	1.44 (3.4)	NA (NA)	3.42	NA	0.8	NA
	Total	660	94	1.74 (3.9)	8.7 (102.7)	4.13	10.8	5.4	24.6

Table 2.9 Sample sizes, standardized abundance and density (standardized numbers were calculated by multiplying mean abundance or density by 1/detection rate, based on respective detection rates) of *Anisakis simplex* s.l. in the musculature of Atlantic cod (*Gadus morhua*) sampled from various areas surrounding Newfoundland and Labrador, and the Flemish Cap during 1947-1953 (Templeman et al. 1957), 1985-1987 (Brattey and Bishop 1992) and 2012-2013. Sampling areas identified below correspond to those described in Figure 11 and Table IV of Templeman et al. (1957) (Appendices C and D), and reference numbers correspond to Fig. 2.2. This table includes only fish ≥ 41 cm and only the fillets (napes were excluded). A = 1947-1953, B = 1985-1987, C = 2012-2013.

	No. of fish			Standardized abundance			*Standardized density		
Sampling area – NAFO Division (reference number)	A	B	C	A	B	C	A	B	C
NE coast Nfld - offshore 3K (1)	699	4093	69	0.02	0.07	0.11	0.05	0.07	0.14
N Grand Bank - 3L W of long 50°W (2)	1004	394	33	0.02	0.05	0.19	0.02	0.05	0.22
Flemish Cap - 3M (3)	357	0	80	0.03	NA	0.22	0.05	NA	0.28

Sampling area – NAFO Division (reference number)	No. of fish			Standardized abundance			*Standardized density		
	A	B	C	A	B	C	A	B	C
SE Grand Bank - 3N (4)	1550	246	94	0.02	0.02	0.16	0.02	0.02	0.19
SW Grand Bank - 3O (5)	922	786	35	0.05	0.07	0.21	0.07	0.12	0.49
St. Pierre Bank S of lat 46°30'N - 3P (6)	344	253	150	< 0.02	0.10	0.22	0.02	0.10	0.45
S coast Nfld, Cape St. Mary's to Pass Island - 3P (7)	736	1142	41	0.02	0.12	0.33	0.02	0.19	0.74
S coast Nfld, Pass Island to Cape Ray - 3P (8)	935	640	86	0.07	0.45	0.35	0.10	0.76	0.99
S section of Nfld W coast, S of Portland Creek - 4R (9)	599	0	47	0.05	NA	0.18	0.12	NA	0.30

* Density is calculated as the total nematodes/kg from all fish combined. This was done to adhere to Templeman et al. (1957) as raw data were not available.

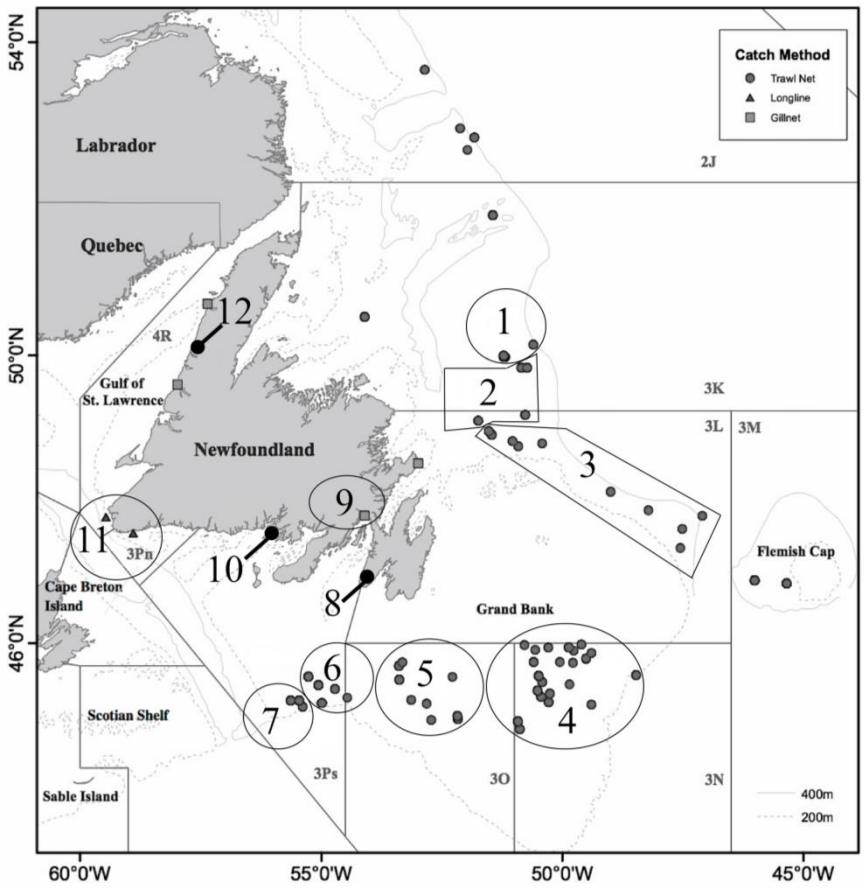

Figure 2.1 Study area indicating NAFO Division boundaries and locations where Atlantic cod were sampled during 2012-2013. Numbers and points surrounded by shapes indicate where samples were grouped for the comparison of nematode infection levels corresponding to Figure 1 of Brattey and Bishop (1992; Appendix B). 1 = Southern Funk Island Bank, 2 = Northeast Newfoundland Shelf, 3 = Grand Bank, 4 = Southeast Shoal of the Grand Bank, 5 = Whale Bank, 6 = Green Bank, 7 = Southern St. Pierre Bank, 8 = Cape St. Mary's, 9 = Placentia Bay, 10 = Pass Island, 11 = Rose Blanche Bank, 12 = Portland Creek. Each point indicates a location where multiple fish were collected (Appendix A). Any samples not encircled were not used in the comparison to historical data from Brattey and Bishop (1992).

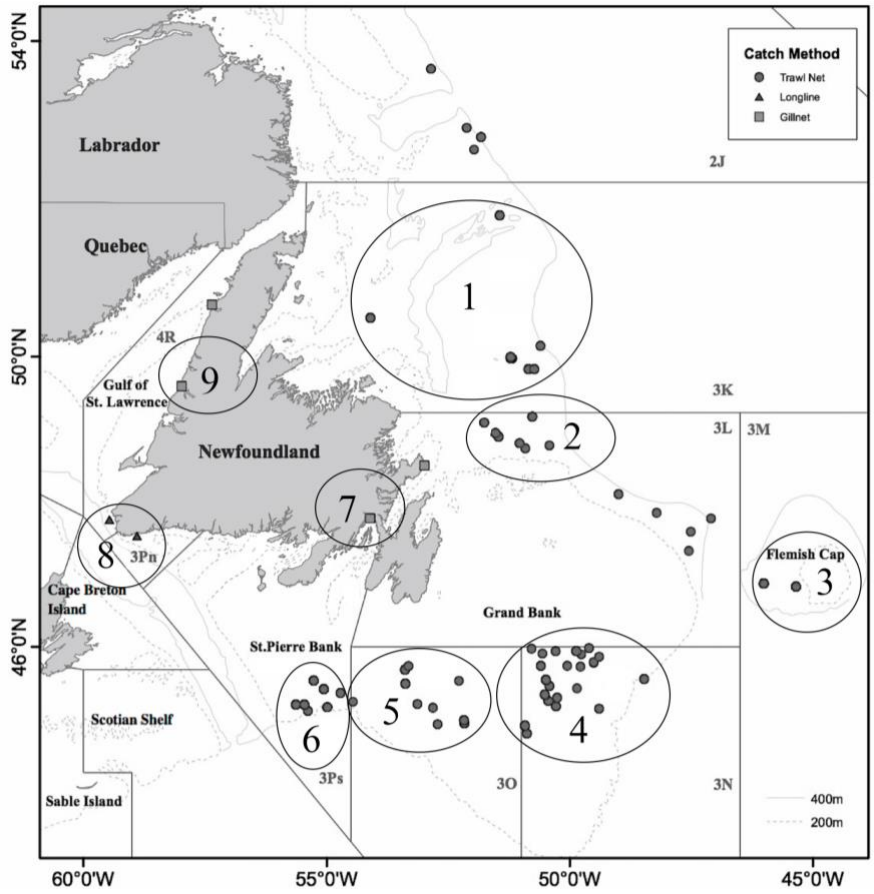

Figure 2.2 Study area indicating NAFO Division boundaries and locations where Atlantic cod were sampled during 2012-2013. Numbers surrounded by shapes indicate where samples were grouped for the comparison of nematode infection levels corresponding to Figure 11 and Table IV of Templeman et al. (1957; Appendices C and D). 1 = Northeast coast of Newfoundland, 2 = Northern Grand Bank (west of long 50° W), 3 = Flemish Cap, 4 = Southeast Grand Bank, 5 = Southwest Grand Bank, 6 = St. Pierre Bank (south of lat 46°30’ N), 7 = Cape St. Mary’s to Pass Island, 8 = Pass Island to Cape Ray, 9 = South of Portland Creek. Each point indicates a location where multiple fish were collected (Appendix A). Any samples not encircled were not used in the comparison to historical data from Templeman et al. (1957).

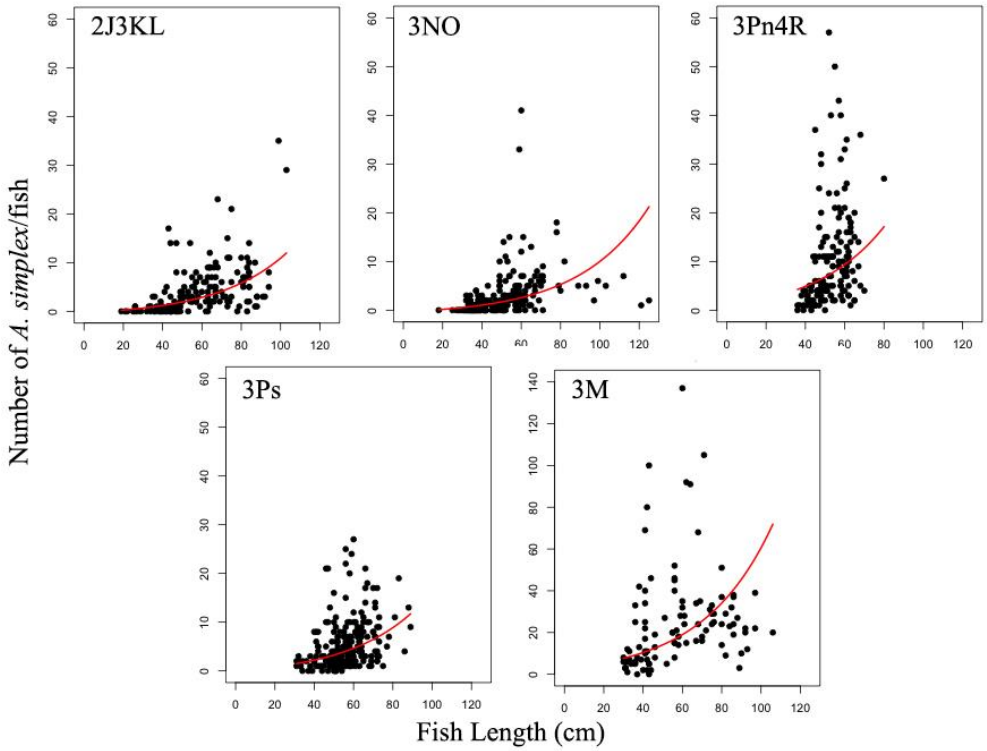

Figure 2.3 Scatter plots of the relationship between number of *Anisakis simplex* s.l. per fish in the musculature and length (nearest cm) of Atlantic cod (*Gadus morhua*) from five cod stocks around Newfoundland and Labrador and the Flemish Cap. Solid line indicates the predicted number of nematodes from the best fitting linear mixed-effects model, see text for details. Note the change in the y-axis for Division 3M.

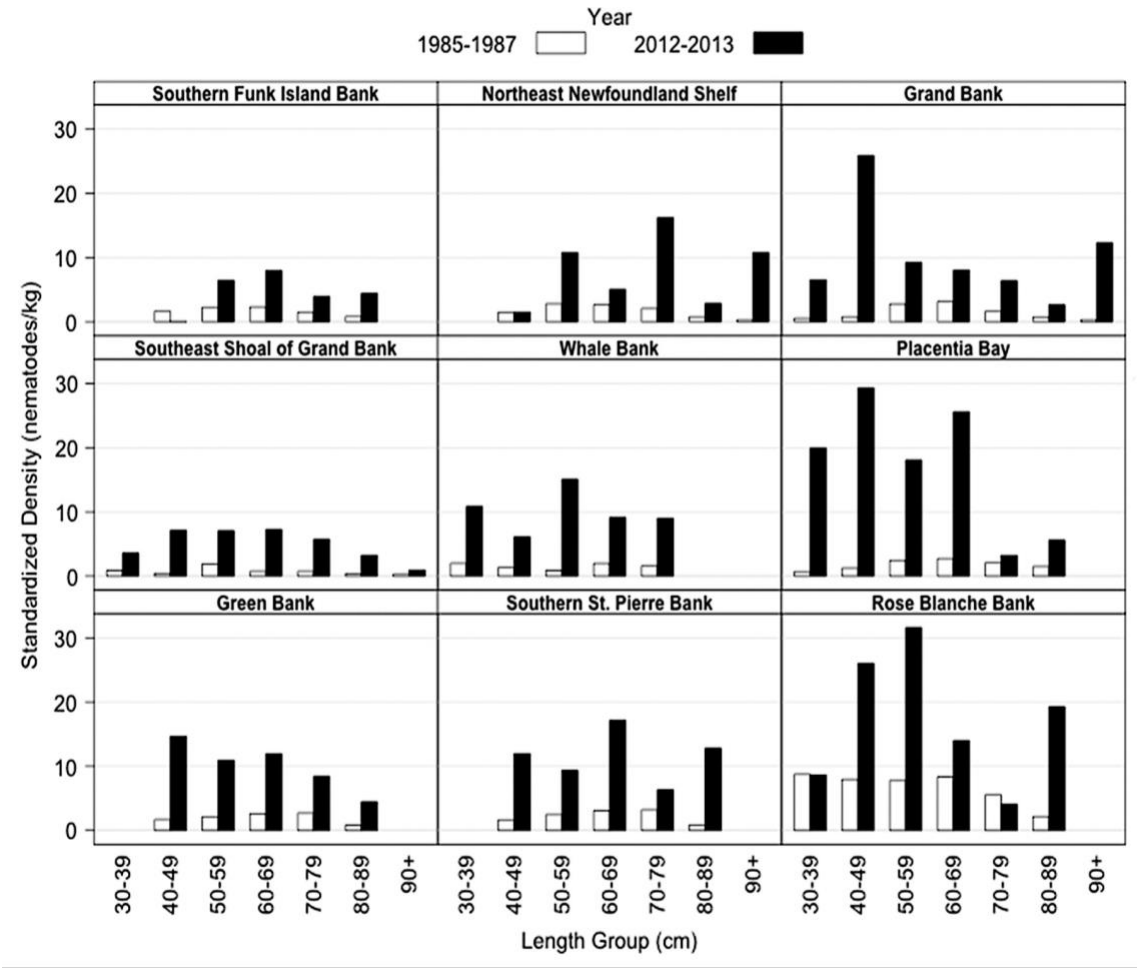

Figure 2.4 Mean standardized density (number of nematodes/kg of musculature) of *Anisakis simplex* s.l. in the musculature of Atlantic cod sampled from various areas off Newfoundland and Labrador during 1985-1987 (Brattey and Bishop 1992) and 2012-2013. Standardized numbers were calculated by multiplying the mean density by 1/detection rate, based on respective detection rates.

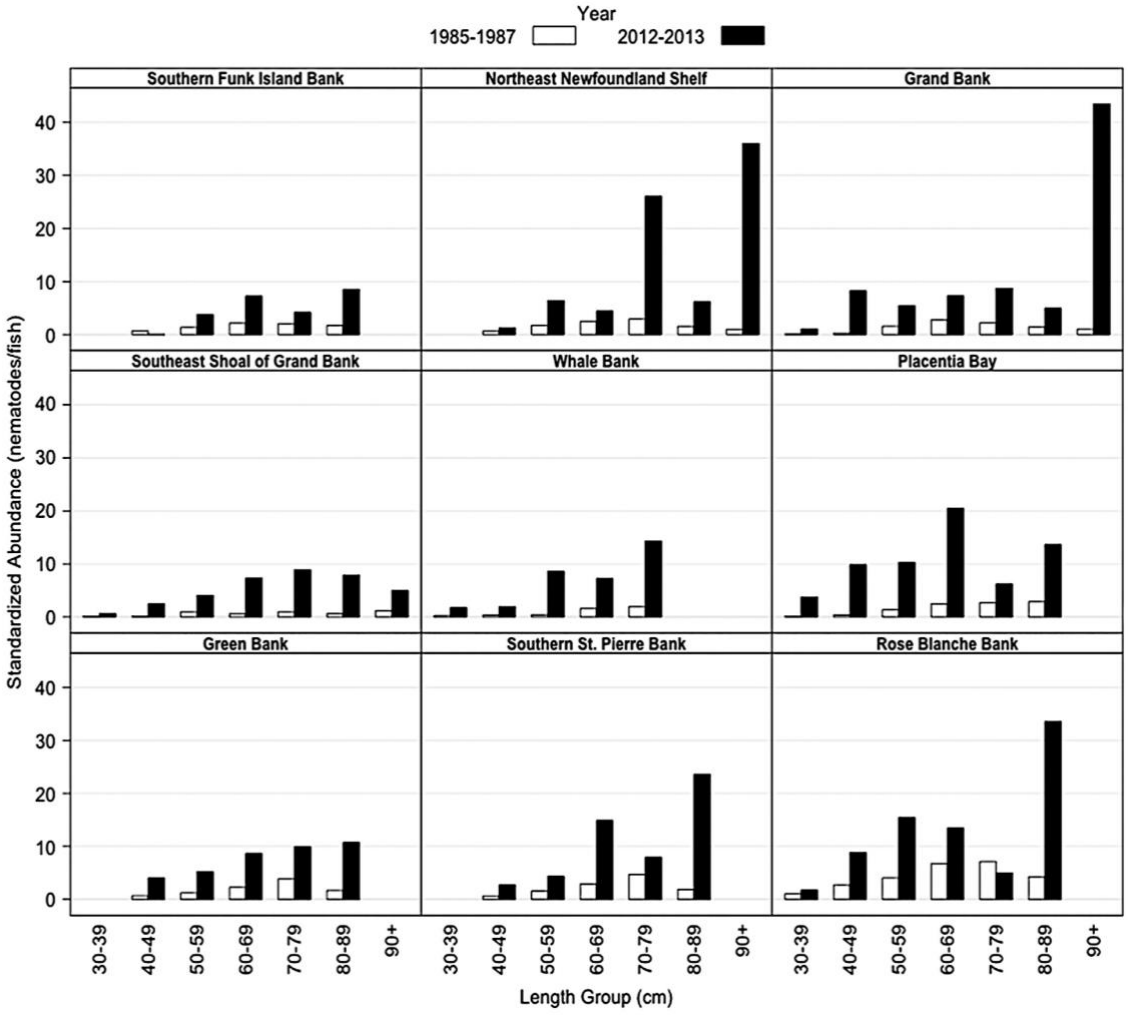

Figure 2.5 Mean standardized abundance (nematodes/fish) of *Anisakis simplex* s.l. in the musculature of Atlantic cod sampled from various areas of Newfoundland and Labrador during 1985-1987 (Brattey and Bishop 1992) and 2012-2013. Standardized numbers were calculated by multiplying the mean abundance by 1/detection rate, based on respective detection rates.

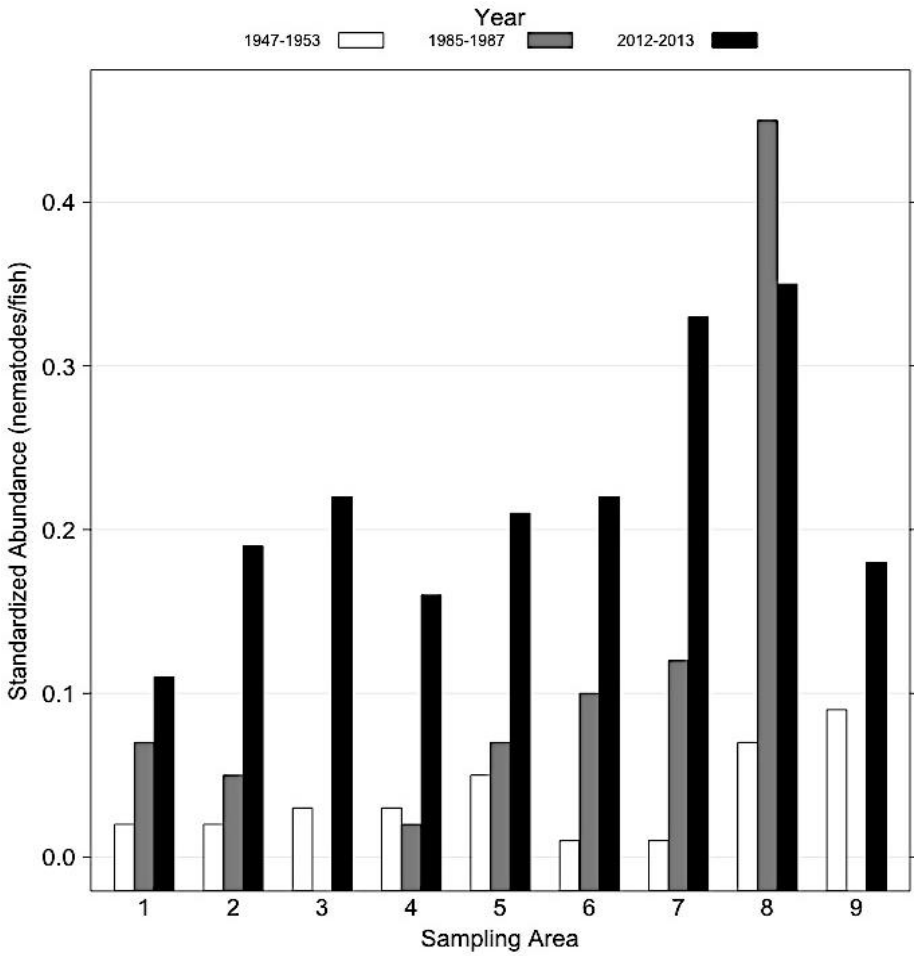

Figure 2.6 Mean standardized abundance (nematodes/fish) of *Anisakis simplex* s.l. in the musculature of Atlantic cod sampled from various areas off Newfoundland and Labrador, and the Flemish Cap during 1947-1953 (Templeman et al. 1957), 1985-1987 (Brattey and Bishop 1992) and 2012-2013. Only Atlantic cod ≥ 41 cm and only nematodes found in the fillets (not the napes) were included in these analyses. 1 = Northeast Coast of Newfoundland, 2 = Northern Grand Bank (west of long 50° W), 3 = Flemish Cap, 4 = Southeast Grand Bank, 5 = Southwest Grand Bank, 6 = St. Pierre Bank (South of lat 46°30' N), 7 = Cape St. Mary's to Pass Island, 8 = Pass Island to Cape Ray, 9 = South of Portland Creek. Where bars are missing, this indicates that data were not collected in this

area for the respective study. Standardized numbers were calculated by multiplying the mean abundance by 1/detection rate, based on respective detection rates.

3 Infection levels of larval *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) in the musculature of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador and on the Flemish Cap

3.1 Abstract

Recent reports from the fishing industry have suggested that there are increasing numbers of parasitic nematodes in some Canadian Atlantic cod (*Gadus morhua*) stocks. These nematodes can pose aesthetic and economic concerns for the fishing industry, particularly if fish are infected with larvae of sealworm (*Pseudoterranova decipiens*). This large and darkly colored ascaridoid nematode resides primarily in the flesh of fish, decreases the appeal of the product and may also be pathogenic to humans when infected fish are eaten raw or improperly cooked. The musculature of 811 Atlantic cod from five cod stocks in the Northwest Atlantic off Newfoundland and Labrador, and on the Flemish Cap were examined for larval *P. decipiens* s.l. I evaluated the distribution of this nematode within the musculature of Atlantic cod, and investigated whether prevalence, abundance, or density varied among cod stocks or in relation to fish length. This study used similar examination techniques to previous studies conducted in the same general areas during 1947-1953 and 1985-1987. In most areas, primarily offshore, the levels of infection were fairly light, typically < 0.40 nematodes/fish. The area with the highest abundance of *P. decipiens* s.l. (> 17 nematodes/fish) was the 3Pn4RS cod stock off western Newfoundland. Although many areas showed no increase in infection levels of *P. decipiens* s.l. since previous studies, there were substantial increases for the west and

south coasts of Newfoundland. The increases in prevalence and abundance of *P. decipiens* s.l. in Atlantic cod in those areas may be due to the increasing grey seal (*Halichoerus grypus*) populations, one of the main definitive hosts of *P. decipiens* s.l. This study also shows that *P. decipiens* s.l. has been remarkably resilient and maintained a widespread distribution despite dramatic ecosystem changes observed over the past three decades. Future researchers should carefully determine and report the detection efficiencies of their methodologies so that infection levels can be compared among studies.

3.2 Introduction

Pseudoterranova decipiens s.l., commonly known as sealworm, is a parasitic nematode whose third larval stage (L3) infects marine fishes and has been reported from more than 75 species within the North Atlantic (Margolis and Arthur 1979; McClelland et al. 1990; McDonald and Margolis 1995; McClelland 2002). It has received much attention in recent decades as fish hosts include several commercially important species such as Atlantic cod (*Gadus morhua*; Templeman et al. 1957; Chandra and Khan 1988; Brattey et al. 1990; Hauksson 2011; Mehrdana et al. 2014; Lunneryd et al. 2015), American plaice (*Hippoglossoides platessoides*; Bristow and Berland 1992; Martell and McClelland 1995), and Yellowtail flounder (*Pleuronectes ferrugineus*; Martell and McClelland 1995). Although larvae can be killed by thorough freezing or cooking (Margolis 1977; McClelland 2002), the presence of *P. decipiens* s.l. in fish products reduces their market value, while detection and removal of the parasites increases cost of production. As a result, *P. decipiens* s.l. represents a major problem for the fishing

industry, costing tens of millions of dollars per annum (Malouf 1986; McClelland 2002). Furthermore, humans may develop a zoonotic disease called pseudoterranovosis which develops when an anisakid nematode, such as *P. decipiens* s.l., infects humans due to eating fish that is either consumed raw or improperly cooked.

Parasitological studies of commercial fishes in Atlantic Canada have revealed significant spatial and temporal variation in levels of larval *P. decipiens* s.l. infection. Extensive surveys conducted during the late 1940s and early 1950s found fish within the southern Gulf of St. Lawrence and around Cape Breton Island to have the highest levels of infection (Scott and Martin 1957; Templeman et al. 1957). For example, Templeman et al. (1957) reported abundance of larval *P. decipiens* s.l. in fillets of Atlantic cod (≥ 41 cm) ranging from 1-2 nematodes per 100 fish in offshore regions east of Newfoundland to 311 nematodes per 100 fish in the southwestern Gulf of St. Lawrence. High parasite abundance within the Gulf of St. Lawrence at the time was attributed to the presence of both grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) which are the principal definitive hosts for *P. decipiens* s.l. (Stobo et al. 1990; Brattey and Stenson 1993).

Subsequent studies during the 1980s showed increased prevalence and abundance of *P. decipiens* s.l. in fish on the Scotian Shelf, the Gulf of St. Lawrence, and along the south coast of Newfoundland (McClelland et al. 1983a; 1983b; 1985; 1987; Brattey et al. 1990). Again, trends in parasite abundance were linked to the abundance of seals, particularly grey seals, which had significantly increased abundance since the early 1960s (Zwanenburg and Bowen 1990; DFO 2017b). Similar associations between infection of cod with *P. decipiens* s.l. larvae and distribution of grey seals have also been reported in

Norway (Jensen et al. 1994), Iceland (Hauksson 2011) and Norway (Mehrdana et al. 2014; Lunneryd et al. 2015).

The increase in Atlantic Canadian grey seals from just a few thousand individuals during the early 1960s to over 400,000 in 2016 (DFO 2017b) has raised questions within the fishing industry about potential impacts on fishery resources, particularly Atlantic cod (DFO 2011). In addition to existing concerns about the effects of sealworm on fish market value and processing cost, it has been hypothesized that grey seals and this parasite may have negative impacts on productivity and even long term viability of some local cod stocks (Chouinard et al. 2005). While grey seals may directly impact some cod stocks through predation (Swain et al. 2011), there is also potential for indirect negative impacts via parasite-induced mortality which may cause damage to organs and tissues, deplete energy reserves, or increase susceptibility of heavily infected fish to predators (McClelland et al. 2011). Between 1962 and 1992, the number of cod in Canadian waters is estimated to have declined by more than 90% (Hutchings and Rangeley 2011) through a combination of over-fishing that began in the 1960s and poor environmental conditions during the late 1980s and early 1990s (Rose 2007; Lilly et al. 2013). With some Newfoundland and Labrador cod stocks showing signs of improvement since the mid 2000s (Rose and Rowe 2015; DFO 2015; DFO 2016; DFO 2017a), and ongoing fisheries in the southern Newfoundland stock in Subdivision 3Ps and the northern Gulf of St. Lawrence stock in Divisions 3Pn4RS, it highlights the need to obtain updated information on the infection levels of *P. decipiens* s.l.

The objective of the present study was to quantify levels of *P. decipiens* s.l. in Atlantic cod stocks surrounding Newfoundland and Labrador for comparison with similar

parasitological surveys conducted during 1947-1953 (Templeman et al. 1957) and 1985-1987 (Brattey et al. 1990). Specifically, by examining 811 Atlantic cod collected from five separate stocks during 2012-2013, I evaluated: i) the distribution of larval *P. decipiens* s.l. within the musculature of Atlantic cod; ii) whether prevalence, abundance, and density of larval *P. decipiens* s.l. within the musculature of Atlantic cod varied in relation to fish size or among stocks; and iii) whether there have been changes in the numbers of larval *P. decipiens* s.l. within the musculature of Atlantic cod since 1947-1953 and 1985-1987.

3.3 Materials and methods

To quantify prevalence and abundance of larval *P. decipiens* s.l. in the musculature of Atlantic cod, samples of whole fish were obtained during 2012 and 2013 from offshore bottom trawl surveys carried out by Fisheries and Oceans Canada and the Centre for Fisheries Ecosystems Research of Memorial University. Stocks and areas sampled included the northern cod stock in Northwest Atlantic Fisheries Organization (NAFO) Divisions 2J3KL, the southern Grand Bank stock in Divisions 3NO, the Flemish Cap stock in Division 3M, and the southern Newfoundland stock in Subdivision 3Ps (Fig. 3.1). Additional samples were obtained from inshore sentinel research fishing activities conducted by local harvesters using long lines and gill nets (stocks and areas sampled included the northern cod stock in Division 3L, the northern Gulf of St. Lawrence stock in Divisions 3Pn4R, and the southern Newfoundland stock in Subdivision 3Ps; Fig. 3.1). Multiple sites were sampled within each of the stock areas to account for potential spatial heterogeneity in nematode infection levels. Cod size structure varied among areas but I

endeavoured to sample as wide a size range as possible within each area to allow for comparisons of infection levels among length classes.

Upon capture, all cod samples were labeled and frozen for detailed examination in the laboratory. To facilitate comparison of results with those obtained during previous studies, the methodology of Templeman et al. (1957) and Brattey et al. (1990) was duplicated for the examination of cod, and identification and removal of nematodes. After being thawed in the lab, the fork length (from the tip of the snout to the end of the middle caudal fin rays) of each individual fish was measured to the nearest centimetre. To quantify the abundance of parasites within the musculature, flesh on the left and right sides of the fish was removed using a filleting knife. The fillets (the dorsal musculature anteriorly and both the dorsal and ventral musculature posteriorly) were separated from napes, or 'belly flaps' (the hypaxial musculature that surrounds the body cavity) by cutting where the ribs join the spinal cord and extending the cut posterioventrally to a point approximately at the middle of the anal fin. Left and right fillet, and nape from each fish were skinned, weighed to the nearest gram, and examined for nematodes on a candling light table (Maritime Plastics, St. John's, NL). Musculature was sliced diagonally into strips approximately 3-5 mm thick to reveal nematodes potentially deeply embedded in the flesh. Areas where skin remained on the musculature or bruising occurred that hindered light passage were examined more thoroughly. Identified nematodes were extracted and preserved in a 1:9 solution of glycerin to 70% ethyl alcohol. Although another parasitic nematode, *Anisakis simplex* s.l., may be found within the musculature of Atlantic cod (Brattey and Bishop 1992), distinct differences in colour, size, and morphology between these two species (Templeman et al. 1957) made

identification by visual inspection possible in most instances. However, in cases of uncertainty, identification was verified by examining specimens under a binocular dissecting microscope.

To determine the efficiency of the examination procedure in detecting nematodes in the musculature, the flesh of 53 randomly selected cod that had previously been examined for nematodes using the candling technique were re-inspected using a pepsin-hydrochloric acid (HCl) digestion technique (Stern et al. 1958) which recovers almost 100% of nematodes present (Brattey 1988), including any that might have been missed during candling and slicing (Brattey et al. 1990). The musculature of each previously examined fish was placed in a 4 L beaker with a mixture comprised of 1 L of warm water, 15 mL of 2N HCl (100%), and 2.5 g of pepsin for each 250 g of flesh. Beaker contents were stirred occasionally to facilitate digestion and after approximately 20 hours, filtered through a 400 µm sieve, rinsed with water, and thoroughly examined in a clear tray over a black background with an ultraviolet light to aid in nematode detection. Again, identified nematodes were extracted and preserved as previously described for subsequent species verification.

Terminology for nematode parasite infection statistics followed Margolis et al. (1982), whereby prevalence is the percentage of cod infected, abundance is the mean number of *P. decipiens* s.l. per cod including uninfected fish, density is the mean number of *P. decipiens* s.l. per kilogram of flesh including uninfected fish, and mean intensity as the mean number of *P. decipiens* s.l. per cod, including only infected individuals.

3.4 Statistical methods

A summary table of infection statistics for *P. decipiens* s.l. was initially prepared in order to present preliminary information regarding intensities of infection and consider the variables that would be of importance while analysing the data (Table 3.1). Comparable summary tables were presented by previous authors examining infection of *P. decipiens* s.l. in the musculature of Atlantic cod (Templeman et al. 1957; Brattey et al. 1990; Boily and Marcogliese 1995; McClelland et al. 2011).

To evaluate larval *P. decipiens* s.l. distribution within the musculature of Atlantic cod, samples from all cod stocks were grouped into eight size groups based on 10-cm increments in fork length from < 30 cm to ≥ 90 cm.

Standard statistical testing assumes that samples are independently and randomly selected. Because fish were collected using gillnets or trawls where a large number of samples are drawn from the same location, this assumption of random sampling could not be met. To address the possibility of heterogeneity of infection rates among the sets within regions, *stock* and *length* were included as fixed-effects, and 'set' as a random effect within the models for analyzing both binomial data (infected vs. uninfected) and count data. To account for zero-inflated data and overdispersion, a hurdle model was used to first analyze the binomial occurrence of nematodes, and subsequently to analyze the total counts of nematodes (Chipeta et al. 2014; Zeileis et al. 2008). The hurdle model consists of two components: an analysis of prevalence followed by an analysis of abundance after removing uninfected fish. Prevalence data were fit using a generalized linear mixed-effects model from the lme4 package in R (Bates et al. 2013). Subsequently, abundance data of infected fish (uninfected fish were removed) were analyzed using a

linear mixed-effects model of the log-transformed count data from the nlme package in R (Pinheiro et al. 2015). All fish from stock 3M were removed due to the small sample size (3 infected fish out of a total 105). To account for the different variance structures among the stocks, the nlme package in R was used with a variance function specification. Both models allow for both fixed and random effects within an unbalanced design, the latter allowing for different variance structures. The Akaike Information Criterion (Akaike 1973) was used to determine the model of best fit for both binomial and count data.

Within the stocks, there was a large degree of deviation within the variances for the abundance data and therefore the model included a specification to allow for different variance structures. Also, most size groups had much greater variance than the mean, indicating that the distribution of nematode counts was highly aggregated within certain individuals, and was not randomly dispersed. For example, the highest number of nematodes found within one fish was 156 within a 68-cm long individual caught in stock 3Pn4R, therefore a log transformation was necessary to normalize the residuals. The addition of 1 to x was not necessary as all counts were above 0, because uninfected fish were removed prior to the analysis.

Pseudoterranova decipiens s.l. recovered from the musculature of Atlantic cod were grouped based on location found within the fish, either the left or right sides of the nape or fillet. G-tests of heterogeneity were conducted to determine if the null hypothesis that the number of nematodes did not differ between the left and right sides, or between the nape and fillet, was correct. The RVAideMemoire package in R was used to perform the G-tests (Hervé 2015).

To investigate whether the number of *P. decipiens* s.l. detected by candling was related to fork length, I calculated the Pearson product-moment correlation coefficient.

To analyze decadal changes in *P. decipiens* s.l. infection levels, samples collected in 2012 and 2013 were grouped according to regions outlined in Figure 1 by Brattey et al. (1990; Fig. 3.1; Appendix B) and Figure 11 and Table IV by Templeman et al. (1957; Fig. 3.2; Appendices C and D); area names were given based on nearest landmarks. Some regions had too few samples for a meaningful comparison and therefore, only regions with ≥ 20 samples were included in the analyses. Bar charts illustrating standardized abundance and density were created using the lattice package (Sarkar 2008) in R statistical software to compare levels of infection of *P. decipiens* s.l. between the historical data and the data collected in the current study (Figs. 3.3 and 3.4). R statistical software was used for all data analyses (R core team 2014) and significance was assessed at $\alpha = 0.05$.

3.5 Results

3.5.1 Summary of infection statistics by geographic region

Pseudoterranova decipiens s.l. was found in all cod stocks surveyed off Newfoundland and Labrador and on the Flemish Cap, although infection levels varied widely; the maximum intensity was 156 nematodes observed in a single fish measuring 68 cm in fork length from off the west coast of Newfoundland (Table 3.1). Prevalence was highly variable among size groups within stocks and ranged from 0% in stocks 2J3KL, 3M and 3NO to 100% in stock 3Pn4R (Table 3.1).

Overall, abundance data showed a similar geographic pattern to prevalence where large differences were observed among the regions surveyed, ranging from 0 nematodes/fish in small cod in stocks 2J3KL, 3M and 3NO to 17 nematodes/fish (variance = NA) in larger fish in stock 3Pn4R (Table 3.1). The lowest abundance was observed in stock 3M, with only one nematode detected in fish < 80 cm in fork length, and within the length classes 80 to ≥ 90 cm, there were two nematodes detected, and the maximum intensity was 1 nematode (Table 3.1). Stocks 2J3KL and 3NO also had very low abundances, with all length classes harbouring < 1 nematode/fish, with the exception of length classes < 30 cm (1.60 nematodes/fish) and ≥ 90 cm (3.86 nematodes/fish) in 3NO (Table 3.1). Stock 3Ps had slightly higher abundances (0.43-2.60 nematodes/fish); however infection levels were highest in fish collected from 3Pn4R (1.29-17.00 nematodes/fish; Table 3.1).

Mean density followed the same trend as abundance and prevalence. However, in most areas, densities peaked in fish of intermediate size. Mean density for the most heavily infected area, stock 3Pn4R, ranged from 4 nematodes/kg (variance = NA) in the 80-89 cm length class to 17 nematodes/kg (variance = 610) in the 40-49 cm length class (Table 3.1). With the exception 3M, the more lightly infected stocks (3Ps, 3NO, and 2J3KL) still harboured moderate densities of nematodes, overall ranging from 0 to 1.8 nematodes/kg, excluding < 30 cm fish in 3NO, which had a mean density of 17 nematodes/kg (Table 3.1). Atlantic cod in 3M harboured fewer nematodes, with 0.03 nematodes/kg over all length classes (Table 3.1).

3.5.2 Statistical modelling of prevalence and abundance data

To further analyze the infection levels of *P. decipiens* s.l. within the musculature of Atlantic cod, a two-component hurdle model was used. The first model, investigating observed changes of prevalence (infected vs. uninfected) data, used a generalized linear model with a binomial error structure and logit link function. The model of best fit included *stock* and *length* as explanatory variables (AIC = 682; Table 3.2). However, the fit was only marginally better than the model that included also the interaction term (AIC = 687).

In this study, the variability in prevalence of *P. decipiens* s.l. among trawl sets was accounted for by introducing a trawl-set specific random effect in the model. The random effect variance would approach zero if there was no variation in parasite numbers among sets and if the data were taken from a homogeneous distribution, or would be a high positive value if most parasites were found in a small proportion of the trawl sets. The random effect variance in this analysis was estimated at 0.37 (SD = ± 0.61) indicating that although variability among sets was low it was not zero and therefore, was accounted for in the model.

The fixed-effect parameter estimates from the generalized linear mixed-effects model [Equation 3] are provided in Table 3.3. In this analysis, the fixed estimates are essentially the intercept terms, and their significance values in Table 3.3 indicate the probability that they are significantly different from zero on a logit scale (= 0.5 or 50% prevalence on a probability scale). When the intercept terms are transformed to a probability scale, they become the baseline prevalence of infection for a fish of 0 cm in length (i.e the intercept on the y-axis). These values show that 3M had the lowest probability of *P. decipiens* s.l.

infection, followed by 2J3KL and 3NO (Table 3.3). Stock 3Ps had slightly higher prevalences, and 3Pn4R had the highest prevalence (Table 3.3). Table 3.3 also shows that stocks 3M and 3Pn4R had non-significant intercept values ($p = 0.54$ and $p = 0.16$, respectively) relative to a prevalence of 0.5, with 3M well below 50% prevalence, and 3Pn4R above 50%. The β term in the model captures the extent to which prevalence increases in length within each stock [Equation 3].

An example is given below to illustrate how predicted prevalence is calculated using the parameters from the modelled relationship between the stock estimate and fork length. In this example, a 50-cm fish from stock 3Pn4R was used and parameter estimates are taken from Table 3.3. The equation is:

$$y = e(\Theta_{S5} + (Y_L * \beta)) / (1 + e(\Theta_{S5} + (Y_L * \beta)) \quad [3]$$

$$y = e(0.81 + (50 * 0.02)) / (1 + e(0.81 + (50 * 0.02))$$

$$y = 0.998$$

Where, y = probability of *P. decipiens* s.l. infection

Θ_S = Stock effect estimate from the generalized linear mixed-effects model

S = Stock (1 = 2J3KL, 2 = 3NO, 3 = 3M, 4 = 3Ps, 5 = 3Pn4R)

Y_L = Length of fish in centimeters

β = Length effect estimate from the generalizes linear mixed-effects model

Hence the model [Equation 3] predicts a 99.8% probability of *P. decipiens* s.l. infection in a 50-cm fish from the 3Pn4R stock. By comparison, the observed data show

that cod between 40-49 cm had a prevalence of 92.2%, and cod between 50-59 cm in stock 3Pn4R had a prevalence of 92.9% (Table 3.1). I also attempted to overlay the model predicted values on scatter plots of the prevalence for each stock, however as the prevalence data are binomial with values of 0 or 1, the plots were not informative as too many points were superimposed on each other. I also calculated prevalence values for each 1-cm length class and overlaid the model predicted estimates through those values; however, I found this approach somewhat misleading as the trends in prevalence with length for some stocks were obscured by small sample sizes for many of the 1 cm length classes. In addition, the model is actually fitting to the raw data, not the values shown in these plots, which could also be misleading. There is no plausible way to plot prevalence as a function of length to clearly illustrate how well the model fits. Consequently, I simply compared my model predicted values with the combined length class values in Table 3.1.

Following the analyses of prevalence of *P. decipiens* s.l., the uninfected fish were removed from the data set and subsequent analyses were conducted on the log transformed count data using a normal error structure with the identity link. Although the AIC values for models which include both *stock* and *length* as well as the interaction terms were slightly lower, they were not significant ($p = 0.0822$ and 0.1161). The model of best fit was therefore the linear mixed-effects model of log transformed counts of *P. decipiens* s.l., which included only *stock* as the explanatory variable (AIC = 220; Table 3.4).

The variability in abundance of *P. decipiens* s.l. between trawl sets was accounted for with a trawl-set specific random effect in the model. The random effect was estimated at

0.05 (SD = ± 0.29) indicating that although variability among sets was low it was not zero and therefore, had to be accounted for in the model

Table 3.5 shows the fixed-effect parameter estimates of the linear mixed-effects model for abundance data. Similar to the model for prevalence data, these estimates are on the log scale and when backtransformed to a linear scale, they illustrate the baseline abundance for a fish of 0 cm in length (i.e. the intercept on the y-axis). The highest level of infection was in 3Pn4R (1.80) and tended to decline eastward and farther offshore, towards 3Ps (1.26), 3NO (1.27), as well as northward towards 2J3KL (1.11; Table 3.5).

3.5.3 Distribution within the musculature of Atlantic Cod

The distribution pattern of *P. decipiens* s.l. within the musculature was related to fork length (Table 3.6). Higher percentages of *P. decipiens* s.l. were observed encysted in the fillets compared to the napes in length classes 40-49 cm (76% of nematodes found in fillet) and 50-59 cm (73%) as compared to the larger length classes of 60-69 cm (58%) and 70-79 cm (63%).

There were slightly more *P. decipiens* s.l. found in the left side of both the nape (total 54%) and the fillet (total 58%) portions, although the percent in the left side of the nape tended to decrease as the length of fish increased. This was not the case for the fillet portion, with percentages in the left side remaining fairly consistent among fish length classes. For the pooled data (when nematode counts from all stocks and fish sizes were combined), the difference between left and right sides was not found to be significant for the nape (G = 2.95, DF =1, p = 0.086), but was significant for the fillet (G = 19.82, DF = 1, p = < 0.001). The heterogeneity among size groups between the numbers of *P.*

decipiens s.l. in the left versus right sides was found to be significant in the nape (G = 12.68, DF = 3, p = 0.005), but not the fillet (G = 0.89, DF = 3, p = 0.828), indicating that the distribution of nematodes differs significantly in the nape but remains consistent in the fillet. The percentage of nematodes in the left side of the nape fluctuated between length classes, yielding a wide range of G values (0.22-12.80). The sample sizes (number of *P. decipiens* s.l.) were also much smaller for the nape, so the statistical power of the G-tests in each length class was much lower compared to the fillet.

3.5.4 Detection rate

The musculature of 53 randomly selected cod (mean fork length ± SE = 57 ± 1.7 cm, range 36-106 cm) collected in NAFO Divisions 4R and 3M were re-inspected for *P. decipiens* s.l. using a pepsin-HCl digestion which recovers nematodes potentially missed during candling. This procedure indicated that percentage recovery for *P. decipiens* s.l. during candling and slicing was 98% (Table 3.7). Brattey et al. (1990) reported 81% recovery, thus necessitating that standardized factors be considered when making comparisons of parasite infection levels between studies. Templeman et al. (1957) did not report detection rate, however it is assumed that their efficiency was similar to that of Brattey et al. (1990), because similar techniques were employed and some individuals participated in both studies.

No association existed between nematode detection rate and fish length (r = -0.16, DF = 51, p = 0.245).

3.5.5 Comparison with previous surveys

Brattey et al. (1990) provided the raw data obtained from their survey, but the raw data from Templeman et al.'s (1957) study were not available and therefore, comparisons were limited to the summary of infection statistics presented. In addition, differences in detection efficiency between studies precluded applying standardized factors to nematode counts from individual fish.

Prevalence, abundance, and density from nine areas of the Northwest Atlantic off Newfoundland and Labrador were comparable between samples collected by Brattey et al. (1990) and samples from the present study (Fig. 3.1). Although all comparable regions had previously been included, some sample sizes were not sufficient enough for meaningful comparisons (areas ≤ 20 fish sampled), and were therefore omitted. Brattey et al. (1990) presented a summary table on prevalence, abundance, and density of *P. decipiens* s.l. infection in the musculature of cod within groups of 10-cm increments of fork length. The same parameters were calculated with the samples collected in the current study and were then compared to the results reported by Brattey et al. (1990). In addition to the values utilized in the summary table, additional samples from raw data provided by Brattey et al. (1990) were also included, primarily in the < 30 cm length class. Based on the results of the summary tables, Whale Bank, Placentia Bay, Green Bank, southern St. Pierre Bank and Rose Blanche Bank had higher abundances of *P. decipiens* s.l. in 2012-2013 compared to 1985-1987, with some exceptions in each area that were lower (Table 3.8). The remaining four regions saw increases in *P. decipiens* s.l. infection, in some length classes while others decreased or remained the same, although the abundances were fairly low overall. It must be noted that variances were higher in this

study and certain length classes within regions had few samples from which to draw a meaningful comparison.

Since the detection rates between the two studies were different, a standardization factor was applied to the abundance and density of *P. decipiens* s.l. A standardized number was calculated based on the detection efficiencies of nematodes of each respective study and applied to the mean abundance and mean density obtained from the raw data (mean abundance * (1/detection rate)). The standardization of the data did not change the overall results that five regions saw an increased abundance of nematodes in 2012-2013 while abundance in four regions remained low and did not appear to change.

The standardized mean density of nematodes during the current study appeared much greater than the study conducted in 1985-1987 in Placentia Bay and Rose Blanche Bank (Table 3.8). Green Bank and southern St. Pierre Bank had lightly infected fish in both studies, although density did increase slightly in the 2012-2013 samples. On the southern Funk Island Bank, northeast Newfoundland Shelf, Grand Bank, southeast shoal of the Grand Bank and on Whale Bank, the mean density is at or slightly above 0 nematodes/kg for both studies, indicating that mean density of *P. decipiens* s.l. in the flesh has been consistently low.

The second historical analysis compared *P. decipiens* s.l. infection levels between the current study and an extensive survey reported by Templeman et al. (1957). The samples collected in the current study were allocated as closely as possible to the regions described in Figure 11 and Table IV by Templeman et al. (1957; Appendices C and D). Nine of these regions were comparable for nematodes in the fillets of Atlantic cod of ≥ 41 cm (Fig. 3.2). The values presented for 1947-1953 were taken from Figure 11 and Table

IV in Templeman et al. (1957) (Fig. 3.2; Appendices C and D). As Templeman et al. (1957) only provided summary statistics without variance; statistical analyses of infection were not possible. Because detection efficiencies were not presented, but should not be ignored, the same standardized number as Brattey et al. (1990) was used to adjust the abundance and density for Templeman et al.'s (1957) values. Given that Templeman et al. (1957) only recorded nematodes found within the fillet, a fairly large portion of *P. decipiens* s.l. infesting the flesh were disregarded. As more nematodes are found in the nape of larger fish, the infection levels are more likely to be underestimated in fish > 60 cm.

All nine areas saw an increase in both abundance and density of *P. decipiens* s.l. infection from 1947-1953 to 2012-2013 (Table 3.9). The most marked increases were observed in three areas, two areas on the south coast of Newfoundland (Cape St. Mary's to Pass Island and Pass Island to Cape Ray), as well as the west coast of Newfoundland (south of Portland Creek). Bar charts were again used to illustrate the changes in the standardized abundance of *P. decipiens* s.l. between the survey in 1947-1953 and the current survey (Fig. 3.5). In general, an increasing trend in infection levels was observed among all areas surveyed.

3.6 Discussion

Pseudoterranova decipiens s.l. in Atlantic cod appear to have increased in abundance in the inshore areas of Newfoundland and Labrador since previous surveys were conducted, most noticeably off the west and southwest coasts of insular Newfoundland. Infection levels were consistently lower in both surveys in the more easterly and offshore

regions. *Pseudoterranova decipiens* s.l. is a species complex comprising of five known species in the North Atlantic, only two of these species have been reported in fishes around Newfoundland, *P. decipiens* s.str. and *P. bulbosa* (Brattey and Davidson 1996). The larvae of *P. bulbosa* occur in the viscera, not the flesh, and therefore the species likely found in this study were *P. decipiens* s.str.

Grey seals, and to a lesser extent harbour seals, are the most important species in the transmission of *P. decipiens* s.l. (Brattey and Stenson 1993; Stobo et al. 2002). Grey seal breeding colonies are located in the Gulf of St. Lawrence, around Nova Scotia and on Sable Island (DFO 2017b) and are therefore closest to the cod stocks surveyed off the west and southwest coasts of Newfoundland. Although there have not been any grey seal breeding colonies identified off Newfoundland and Labrador, some individuals are known to migrate to Newfoundland waters to forage, particularly off the west and south coasts (Breed et al. 2009). There are also many small harbour seal breeding concentrations around the coasts of Newfoundland and Labrador, as well as in the Gulf of St. Lawrence and eastern Newfoundland (Hammill et al. 2010). The lower numbers of larval *P. decipiens* s.l. observed in cod towards the east and in offshore areas such as the southern Grand Bank (Divisions 3NO), the northern cod stock (Divisions 2J3KL), and the Flemish Cap (Division 3M), likely reflect the lower numbers of grey and harbour seals in those regions among other factors discussed below. The results show that none of the cod stocks surveyed were completely free of larval *P. decipiens* s.l. but from a processing perspective removal of larval nematodes would not be as labour intensive in cod stocks in 2J3KL, 3NO, and 3M.

Abiotic factors, such as bottom temperatures, may also influence the abundance of *P. decipiens* s.l. Parasitological surveys conducted have suggested that the decreasing abundance of *P. decipiens* s.l. in fishes was attributed to decreasing bottom temperatures (Marcogliese 2001; McClelland et al. 2011). As bottom temperatures drop, it inhibits the hatching, development, and survival of *P. decipiens* s.l. eggs released from the definitive host, and conversely as temperatures increase it has an opposing effect and would result in an increase in abundance (McClelland et al. 2011). The warming trend seen over the past few years in the North Atlantic (Colbourne et al. 2014) may partly explain the increased levels of *P. decipiens* s.l. infection in the flesh of cod.

Caution must be used when comparing the results from surveys of larval *P. decipiens* s.l., as there are often sources of variation between studies (Brattey et al. 1990). Differences in the efficiency of nematode detection may play a role, and are not always reported in larval nematode surveys. It is essential for subsequent larval nematode surveys to report efficiency of nematode detection to allow more accurate comparisons of changes in prevalence and abundance. In this study the same methodology as Brattey et al. (1990) was used, and in both studies the efficiency of detection was determined. The abundance estimates were adjusted with standardized factors using respective detection efficiency values to make comparisons; however this adjustment did not change the overall results. The number of samples and the size grouping of the samples may also be important when comparing survey results. Compared to previous surveys, the present study was less extensive, had smaller sample sizes and cod in the largest size groups were sometimes scarce as some stocks have not recovered their full size and age structure since the collapse of groundfish in the early 1990s. Nonetheless, these issues are addressed

simply by restricting the comparisons to the same length classes. Overall, the survey results are consistent with other biological information; particularly regarding the notable changes in grey seal abundance around Newfoundland and Labrador.

A more recent survey done by Boily and Marcogliese (1995) presented *P. decipiens* s.l. infection statistics from various areas within the Gulf of St. Lawrence. Only one area (southwest coast of Newfoundland in Division 4R, Figure 1 by Boily and Marcogliese 1995) was comparable. However the detection rates were not reported for this study, making direct comparisons more difficult, but based on the presented results in Table 1 of Boily and Marcogliese (1995), the overall prevalence found on the southwest coast of Newfoundland was 20% compared to the 94% found in this study. Overall abundance also increased from 0.45 nematodes/fish in 1992 (Boily and Marcogliese 1995) to 2.83 nematodes/fish in 2012-2013. Compared to Boily and Marcogliese (1995), it appears that abundance increased in all length groups since 1992 as well. However, it must be noted again that it is difficult to determine the accuracy of comparisons without a measure of the detection rates.

In addition to the increasing grey seal populations, climate change and ecosystem changes associated with the collapse of groundfish stocks may also have influenced *P. decipiens* s.l. (Marcogliese 2001; Genovesi et al. 2011; McClelland et al. 2011). This parasite primarily follows a benthic life cycle, as the larvae are not mobile. As they reach the seabed, crustaceans and other invertebrates (first intermediate hosts) consume them. Since the collapse of groundfish and the larger predatory fish in the Northwest Atlantic, there have been major changes in food webs and abundances of various species that may be involved in transmission of *P. decipiens* s.l. (Frank et al. 2005; Shackell et al. 2012).

For example, an increase of certain fishes in the diet of Atlantic cod, such as American plaice (Hanson and Chouinard 2002), which have been reported to harbour substantial numbers of *P. decipiens* s.l. larvae (McClelland et al. 2011), would likely increase the number of nematodes they are exposed to and cause an increase in infection levels. It remains difficult to evaluate specifically how these changes have impacted the transmission of *P. decipiens* s.l. However, the results show that *P. decipiens* s.l. have been remarkably resilient to dramatic ecosystem changes observed over the past three decades and have maintained a widespread distribution and increased in abundance among cod in several areas, although the overall size of many of the cod populations are below their pre-collapse levels.

Although changes in the distribution and abundance of the definitive hosts of these nematodes may be contributing factors, many other factors may be influencing the increasing infection levels observed in this study. In parasitological surveys conducted in the Gulf of St. Lawrence (Boily and Marcogliese 1995; Marcogliese et al. 1996), the decreasing trend in *P. decipiens* s.l. infection in fishes was attributed to the decreasing bottom temperatures which inhibited the hatching and development of the eggs. Conversely, warming trends in the Atlantic Ocean accelerate the hatching times, development and survival of *P. decipiens* s.l. (McClelland et al. 2011) and *A. simplex* s.l. (Brattey and Clark 1992). The warming trend seen over the past few years in the North Atlantic (Colbourne et al. 2014) may partly explain the increased levels of *P. decipiens* s.l. infection in the flesh of cod.

Many parasitological studies conducted worldwide have also determined similar factors influencing an increase in nematode abundance. A study in Iceland concluded that

Atlantic cod caught inshore near the definitive host, the grey seal, haul out sites which coincides with warmer waters than offshore areas, had much higher levels of *P. decipiens* s.l. infection (Hauksson 2011). McClelland et al. (2000) suggest that abundance of *P. decipiens* s.l. in American plaice was correlated to near bottom temperatures as well as proximity to the definitive host.

This study showed that *P. decipiens* s.l. were more commonly found in the left side of the fish, which was also observed in previous studies (Brattey and Bishop 1992; Smith and Hemmingsen 2003). However, I found significant heterogeneity between the numbers of *P. decipiens* s.l. found in the nape, likely due to smaller numbers of nematodes found in the napes of smaller fish. This also coincides with what I observed for *Anisakis simplex* s.l. (see Chapter 2). Smith and Hemmingsen (2003) proposed two hypotheses to explain these results. The first, based on visceral organ topography, is that the left lobe of the liver is much larger than the right and lies against the stomach wall. It is likely that the left lobe of the liver is the first organ encountered, and subsequently, the easiest route to follow would be to enter the flesh on the left side (Smith and Hemmingsen 2003). The second hypothesis, which may act synergistically with the first, is that the larvae have a predilection for the liver. As Smith and Hemmingsen (2003) suggested, it would be beneficial for processing companies to look more closely for nematodes in the flesh on the left side of the fish.

Several studies have shown that the numbers of *P. decipiens* s.l. L3 larvae tend to become more prevalent in the napes compared to the fillets of a fish as the cod size (age) increases, with a greater proportion of larvae present in the fillets of smaller fish as opposed to the napes (McClelland et al. 1990). The same trend was present in the current

study in fish < 79 cm. This may be an evolutionary adaptation by the nematodes to conserve energy, as it would be a much greater distance to migrate to the fillet of a larger fish. In contrast to *P. decipiens* s.l., *Anisakis simplex* s.l. in the flesh of cod is primarily found in the napes (see Chapter 2). The larger size of *P. decipiens* s.l. may allow it to migrate much deeper into the flesh than *A. simplex* s.l.

The viscera of cod were not inspected for *P. decipiens* s.l. larvae as part of this study, as only the nematodes in the flesh were of primary interest to the fishing industry. *Pseudoterranova decipiens* s.l. larvae are less likely to be observed in the viscera of smaller cod as most of them reside in the flesh, however they are more prevalent in the viscera of larger fish (McClelland 2002).

Overall, the number of *P. decipiens* s.l. L3 larvae in the flesh of Atlantic cod appeared to have increased in the inshore areas off Newfoundland in the Northwest Atlantic, particularly in areas closest to grey seal populations, consistent with the increasing abundance of grey seals and increasing bottom temperatures.

Currently, candling is the technique utilized for their removal. The process involves placing the musculature over a white light table where parasites are revealed as dark spots and can be detected by visual inspection. However it is an extremely labour-intensive and inefficient process, with detection efficiencies varying between 33% and 93% (McClelland 2002). Other methods of parasite detection and removal in fillets are being developed, such as imaging spectroscopy (Heia et al. 2007), electromagnetic detection (Choudhury and Bublitz 1994), and sensitive magnetometer detection (Jenks et al. 1996), however candling remains the primary method used by most processing plants (McClelland et al. 2002). In terms of decreasing processing costs, the Newfoundland cod

stocks that are located farther offshore and eastward harbour the lowest infection levels of *P. decipiens* s.l.

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Table 3.1 Infection statistics for *Pseudoterranova decipiens* s.l. in the musculature of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador, and on the Flemish Cap during 2012-2013. Prevalence: percentage of fish infected; Abundance: mean number of nematodes per fish including uninfected fish (nematodes/fish); Maximal abundance: maximal number of nematodes in a single fish; Mean density: mean number of nematodes per kilogram of musculature (skinned nape and fillet) including uninfected fish (nematodes/kg); Mean Intensity: mean number of nematodes in infected fish, excluding uninfected fish (nematodes/infected fish).

	Variables (definitions in caption)						
NAFO Division and dates	Length class (cm)	No. of fish	% Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
2J3KL	< 30	9	0.0 (NA)	0.00 (0.00)	0	0.00	NA
2012: July, November,	30-39	15	20.0 (7.0-45.2)	0.20 (0.17)	1	1.21	1.00
December; 2013: May	40-49	35	11.4 (4.5-26.0)	0.26 (0.67)	4	0.81	2.25

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	% Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
	50-59	27	22.2 (10.6-40.8)	0.26 (0.28)	2	0.44	1.17
	60-69	30	6.7 (1.8-21.3)	0.07 (0.06)	1	0.07	1.00
	70-79	17	29.4 (13.3-53.1)	0.35 (0.37)	2	0.28	1.20
	80-89	23	26.1 (12.5-46.5)	0.39 (0.61)	3	0.19	1.50
	≥ 90	6	33.3 (9.7-70.0)	0.50 (0.70)	2	0.16	1.50
	Total	162	17.3 (12.2-23.8)	0.24 (0.37)	4	0.27	1.39
3M	30-39	24	4.2 (0.2-20.2)	0.04 (0.04)	1	0.33	1.00
2012: May-June; 2013:	40-49	22	0.0 (NA)	0.00 (0.00)	0	0.00	NA
April	50-59	15	0.0 (NA)	0.00 (0.00)	0	0.00	NA
	60-69	13	0.0 (NA)	0.00 (0.00)	0	0.00	NA

NAFO Division and dates	Length class (cm)	No. of fish	% Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
			Variables (definitions in caption)				
	70-79	10	0.0 (NA)	0.00 (0.00)	0	0.00	NA
	80-89	14	7.1 (0.4-31.5)	0.07 (0.07)	1	0.04	1.00
	≥ 90	7	14.3 (0.7-51.3)	0.14 (0.14)	1	0.05	1.00
	Total	105	2.9 (1.0-8.1)	0.03 (0.03)	1	0.03	1.00
3NO	< 30	5	20.0 (1.0-62.4)	1.60 (12.80)	8	16.88	8.00
2012: September-October	30-39	46	15.2 (7.6-28.2)	0.30 (1.46)	8	1.87	2.00
	40-49	42	4.8 (1.3-15.8)	0.05 (0.05)	1	0.14	1.00
	50-59	44	11.4 (5.0-24.0)	0.18 (0.43)	4	0.32	1.60
	60-69	27	3.7 (0.2-18.3)	0.04 (0.04)	1	0.04	1.00
	70-79	9	44.4 (18.9-73.3)	0.56 (0.53)	2	0.36	1.25

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	% Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
	80-89	3	0.0 (NA)	0.00 (0.00)	0	0.00	NA
	≥ 90	7	42.9 (15.8-75.0)	3.86 (24.48)	11	0.70	9.00
	Total	183	12.6 (8.5-18.2)	0.36 (2.15)	11	0.49	2.83
3Ps	30-39	13	30.8 (12.7-57.6)	0.46 (0.60)	2	3.34	1.50
2012: April-May; 2013:	40-49	37	48.6 (33.4-64.1)	1.14 (3.12)	8	3.96	2.33
May	50-59	82	46.3 (36.0-57.1)	1.09 (3.41)	9	2.16	2.34
	60-69	49	24.5 (14.6-38.1)	0.43 (0.88)	4	0.58	1.75
	70-79	21	47.6 (28.3-67.6)	1.00 (2.80)	7	0.81	2.10
	80-89	5	40.0 (11.8-76.9)	2.60 (22.80)	11	1.13	6.50
	Total	207	40.6 (34.1-47.4)	0.93 (3.00)	11	1.51	2.29

			Variables (definitions in caption)				
NAFO Division and dates	Length class (cm)	No. of fish	% Prevalence (95% CI)	Abundance (variance)	Maximal abundance	*Mean density	Mean intensity
3Pn4R	30-39	7	42.9 (15.8-75.0)	1.29 (2.90)	4	6.24	3.00
2012: October; 2013: July-September	40-49	51	92.2 (81.5-96.9)	5.67 (63.75)	42	16.68	6.15
	50-59	56	92.9 (83.0-97.2)	6.18 (62.11)	53	10.21	6.65
	60-69	38	86.8 (72.7-94.2)	10.08 (758.62)	156	10.54	11.61
	70-79	1	100.0 (5.1-100.0)	17.00 (NA)	17	13.87	17.00
	80-89	1	100.0 (5.1-100.0)	7.00 (NA)	7	4.02	7.00
	Total	154	89.0 (83.0-93.0)	6.82 (232.04)	156	11.43	7.67

*Density was calculated for each individual fish (nematodes/kg), then the mean of those densities was calculated within each length class for the Mean Density.

Table 3.2 Comparison of various linear model fits to the binomial data (infected = 1, not infected = 0) for Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and on the Flemish Cap infected with *Pseudoterranova decipiens* s.l. Models were fitted using the logit link function with a binomial error structure. The asterisk (*) indicates an interaction term is included as a parameter in the model. The model of best fit is indicated in bold.

R model					
Fixed-effects	Random effects	DF	AIC	Log likelihood	Deviance
Infected ~ stock + length	**+ (1|set)**	**7**	**682**	**-334**	**668**
Infected ~ stock * size.cm	+ (1|set)	11	687	-333	666
Infected ~ 0 + stock	+ (1|set)	6	690	-339	678
Infected ~ 0 + length	+ (1|set)	2	766	-381	762
Infected ~ 0	+ (1|set)	1	783	-390	781

Table 3.3 Parameter estimates for a generalized linear mixed-effects model [Equation 3] with binomial error structure and log link function fitted to binomial (prevalence) data of *Pseudoterranova decipiens* s.l. within Atlantic cod (*Gadus morhua*) stocks surrounding Newfoundland and Labrador, and on the Flemish Cap. Values in parentheses are stock effect estimates transformed to probability of infection (prevalence).

Fixed-effects	Stock effect estimate	Standard error	z-value	p
Length (β)	0.02	0.01	3.098	0.0020
2J3KL (Θ_{S1})	-3.10 (0.04)	0.54	-5.707	< 0.0001
3M (Θ_{S2})	-5.02 (0.01)	0.89	-5.652	0.5360
3NO (Θ_{S3})	-3.39 (0.03)	0.51	-6.640	< 0.0001
3Ps (Θ_{S4})	-1.74 (0.15)	0.51	-3.410	0.0007
3Pn4R (Θ_{S5})	0.81 (0.69)	0.58	1.415	0.1572

Table 3.4 Comparison of various linear model fits to the log(*x*) transformed count data without uninfected fish (zero counts) for Atlantic cod (*Gadus morhua*) surrounding Newfoundland and Labrador, and on the Flemish Cap infected with *Pseudoterranova decipiens* s.l. Models were fitted using the identity link function with a normal error structure. The asterisk (*) indicates an interaction term is included as a parameter in the model. The model of best fit is indicated in bold.

R model					
Fixed-effects	Random effects	DF	AIC	Log likelihood	p
Log(*x*) ~ 0	+ (1|set)	5	263	-127	
Log(*x*) ~ 0 + length	+ (1|set)	6	243	-115	< 0.0001
Log(*x*) ~ 0 + stock	**+ (1|set)**	**9**	**220**	**-101**	**< 0.0001**
Log(*x*) ~ stock + length	+ (1|set)	10	219	-100	0.0822
Log(*x*) ~ stock * length	+ (1|set)	13	219	-97	0.1161

Table 3.5 Parameter estimates for a linear mixed-effects model of log transformed count data of *Pseudoterranova decipiens* s.l. after the removal of uninfected fish in Atlantic cod (*Gadus morhua*) stocks surrounding Newfoundland and Labrador. Values in parentheses are stock effect estimates back transformed to a linear scale. All fish from stock 3M were removed due to the small sample size (3 infected fish out of a total 105).

Fixed-effects	Stock effect estimate	Standard error	T-value
2J3KL	0.10 (1.11)	0.04	2.79
3NO	0.24 (1.27)	0.08	2.81
3Ps	0.23 (1.26)	0.04	5.64
3Pn4R	0.59 (1.80)	0.05	12.45

Table 3.6 Distribution of *Pseudoterranova decipiens* s.l. in the musculature (left and right nape and fillet) of Atlantic cod (*Gadus morhua*) from stocks off Newfoundland and Labrador, and on the Flemish Cap during 2012-2013. The null hypothesis being tested is that nematodes are equally distributed between left and right sides of nape and fillet portions for four length classes of cod.

		Nape				Fillet				% of Total
Length Class (cm)	No. of fish	No. of *P. decipiens*	% in L. side	G	P	No. of *P. decipiens*	% in L. side	G	P	*P. decipiens* in fillets
40-49	187	83	56.6	1.46	n.s	259	59.8	10.11	**	75.7
50-59	224	121	66.1	12.80	***	329	56.2	5.12	.	73.1
60-69	157	170	45.9	1.15	n.s	237	57.4	5.19	.	58.2
70-79	58	18	44.4	0.22	n.s	31	54.8	0.29	n.s	63.3
Pooled	626	392	54.3	2.95	n.s	856	57.6	19.82	***	
		Total		15.63	**	Total		20.71	***	68.6

		Nape				Fillet				% of Total
Length Class (cm)	No. of fish	No. of *P. decipiens*	% in L. side	G	P	No. of *P. decipiens*	% in L. side	G	P	*P. decipiens* in fillets
		Heterogeneity		12.68	**	Heterogeneity		0.89	n.s	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

Table 3.7 Percentage of *Pseudoterranova decipiens* s.l. recovered from the musculature of Atlantic cod (*Gadus morhua*) using the candling and slicing technique. Total: number of nematodes recovered candling in addition to the number of nematodes subsequently found using HCL digestion.

Length class (cm)	No. of fish	Mean length (cm)	Prevalence (%)	Abundance ± SE	Maximum abundance	Number recovered candling	Total	Recovery rate (%)
35-44	6	41.33	50.0	5.50 ± 5.59	31	33	33	100.0
45-54	18	49.11	88.9	4.67 ± 1.60	29	83	84	98.8
55-64	19	59.42	89.5	12.89 ± 4.33	74	236	245	96.3
65-74	7	67.29	71.4	24.14 ± 23.76	156	168	169	99.4
≥ 75	3	90.67	33.3	2.33 ± 2.86	7	7	7	100.0
Total	53	56.68	79.3	10.15 ± 3.35	156	527	538	98.0

Table 3.8 Sample sizes, mean abundance (variance), standardized mean abundance and standardized mean density (standardized numbers were calculated by multiplying the mean abundance or density by 1/detection rate based on respective detection rates) of *Pseudoterranova decipiens* s.l. in the flesh of Atlantic cod (*Gadus morhua*) from various areas surrounding Newfoundland and Labrador, and on the Flemish Cap during 1985-1987 (Brattey et al. 1990) and 2012-2013. Sampling areas correspond to those in Figure 1 of Brattey and Bishop (1992; Appendix B) and reference numbers correspond to Fig. 2.1.

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
Southern Funk	30-39	0	9	NA (NA)	0.11 (0.11)	NA	0.11	NA	0.67
Island Bank (1)	40-49	172	2	0.09 (0.12)	0.00 (0.00)	0.11	0.00	0.28	0.00
	50-59	758	1	0.11 (0.52)	1.00 (NA)	0.14	1.02	0.22	1.76
	60-69	335	7	0.14 (0.42)	0.00 (0.00)	0.17	0.00	0.19	0.00
	70-79	81	8	0.14 (0.19)	0.38 (0.55)	0.17	0.38	0.12	0.36

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	80-89	21	12	0.19 (0.26)	0.33 (0.42)	0.23	0.34	0.11	0.18
	≥ 90	0	3	NA (NA)	0.67 (1.33)	NA	0.68	NA	0.23
	Total	1367	42	NA (NA)	0.26 (0.34)	NA	0.27	NA	0.23
Northeast	< 30	0	9	NA (NA)	0.00 (0.00)	NA	0.00	NA	0.00
Newfoundland	30-39	13	0	0.08 (0.08)	NA (NA)	0.10	NA	0.57	NA
Shelf (2)	40-49	246	1	0.03 (0.04)	0.00 (NA)	0.04	0.00	0.09	0.00
	50-59	851	9	0.18 (2.52)	0.11 (0.11)	0.22	0.11	0.36	0.19
	60-69	406	7	0.12 (0.79)	0.00 (0.00)	0.15	0.00	0.15	0.00
	70-79	95	1	0.28 (0.52)	1.00 (NA)	0.35	1.02	0.25	0.63
	80-89	17	4	0.41 (2.13)	0.50 (0.33)	0.51	0.51	0.25	0.23
	≥ 90	17	1	0.47 (1.64)	0.00 (NA)	0.58	0.00	0.16	0.00

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	Total	1645	32	NA (NA)	0.13 (0.11)	NA	0.13	NA	0.15
Grand Bank	30-39	29	6	0.00 (0.00)	0.33 (0.27)	0.00	0.34	0.00	2.13
(3)	40-49	70	9	0.16 (0.37)	0.33 (0.50)	0.20	0.34	0.59	1.06
	50-59	178	8	0.08 (0.15)	0.50 (0.57)	0.10	0.51	0.19	0.87
	60-69	122	8	0.09 (0.25)	0.25 (0.21)	0.11	0.26	0.12	0.28
	70-79	86	3	0.28 (0.51)	0.00 (0.00)	0.35	0.00	0.26	0.00
	80-89	32	2	0.28 (0.53)	0.00 (0.00)	0.35	0.00	0.17	0.00
	≥ 90	35	1	0.86 (9.48)	1.00 (NA)	1.06	1.02	0.28	0.29
	Total	552	37	NA (NA)	0.32 (0.34)	NA	0.33	NA	0.45
Southeast	< 30	0	1	NA (NA)	0.00 (NA)	NA	0.00	NA	0.00
Shoal of the	30-39	19	30	0.00 (0.00)	0.17 (0.14)	0.00	0.17	0.00	1.05

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
Grand Bank	40-49	20	24	0.05 (0.05)	0.04 (0.04)	0.06	0.04	0.21	0.12
(4)	50-59	22	33	0.00 (0.00)	0.06 (0.06)	0.00	0.06	0.00	0.11
	60-69	20	20	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00	0.00
	70-79	27	7	0.07 (0.07)	0.29 (0.24)	0.09	0.29	0.07	0.19
	80-89	27	3	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00	0.00
	≥ 90	115	7	0.20 (4.60)	3.86 (24.48)	0.25	3.94	0.05	0.71
	Total	250	125	NA (NA)	0.30 (2.02)	NA	0.30	NA	0.35
Whale Bank	< 30	0	4	NA (NA)	2.00 (16.00)	NA	2.04	NA	39.26
(5)	30-39	31	16	0.00 (0.00)	0.56 (4.00)	0.00	0.57	0.00	3.49
	40-49	29	18	0.00 (0.00)	0.06 (0.06)	0.00	0.06	0.00	0.18
	50-59	55	11	0.07 (0.11)	0.55 (1.47)	0.09	0.56	0.17	0.98

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	60-69	63	6	0.05 (0.08)	0.17 (0.17)	0.06	0.17	0.07	0.21
	70-79	36	2	0.03 (0.03)	1.50 (0.50)	0.04	1.53	0.02	0.96
	80-89	27	0	0.07 (0.07)	NA (NA)	0.09	NA	0.05	NA
	≥ 90	102	0	0.89 (41.05)	NA (NA)	1.10	NA	0.26	NA
	Total	343	57	NA (NA)	0.49 (2.50)	NA	0.50	NA	1.26
Placentia Bay	30-39	22	1	0.23 (0.28)	0.00 (NA)	0.28	0.00	1.72	0.00
	40-49	57	13	0.25 (0.26)	1.85 (6.47)	0.31	1.88	0.99	5.61
	50-59	56	25	0.52 (3.16)	2.60 (5.83)	0.64	2.65	1.14	4.67
	60-69	57	2	0.86 (5.87)	1.00 (0.00)	1.06	1.02	1.17	1.27
	70-79	49	1	2.04 (42.12)	3.00 (NA)	2.52	3.06	1.93	1.57
	80-89	19	1	3.47 (111.93)	11.00 (NA)	4.28	11.23	2.22	4.59

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	≥ 90	13	0	0.77 (39.86)	NA (NA)	0.95	NA	1.69	NA
	Total	273	43	NA (NA)	2.44 (7.30)	NA	2.49	NA	4.33
Green Bank (6)	30-39	0	6	NA (NA)	0.33 (0.67)	NA	0.34	NA	2.50
	40-49	38	15	0.18 (0.97)	0.53 (0.70)	0.22	0.54	0.56	1.97
	50-59	250	51	0.25 (1.20)	0.45 (1.05)	0.31	0.46	0.53	0.97
	60-69	189	47	0.16 (0.26)	0.40 (0.90)	0.20	0.41	0.22	0.57
	70-79	50	15	0.12 (0.23)	0.53 (0.41)	0.15	0.54	0.10	0.46
	80-89	29	3	0.24 (0.40)	0.67 (1.33)	0.30	0.68	0.14	0.28
	≥ 90	29	0	0.83 (4.58)	NA (NA)	1.02	NA	0.30	NA
	Total	585	137	NA (NA)	0.45 (0.85)	NA	0.46	NA	0.72
	30-39	0	6	NA (NA)	0.67 (0.67)	NA	0.68	NA	5.15

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
Southern St.Pierre Bank (7)	40-49	15	9	0.13 (0.12)	1.11 (1.61)	0.16	1.13	0.42	4.91
	50-59	171	6	0.05 (0.30)	0.17 (0.17)	0.06	0.17	0.10	0.37
	60-69	145	1	0.08 (0.23)	0.00 (NA)	0.10	0.00	0.11	0.00
	70-79	59	5	0.02 (0.02)	2.00 (9.50)	0.02	2.04	0.01	1.62
	80-89	9	1	0.33 (1.00)	0.00 (NA)	0.41	0.00	0.19	0.00
	Total	399	28	NA (NA)	0.89 (2.47)	NA	0.91	NA	1.74
Rose Blanche Bank (10)	30-39	18	7	0.22 (0.18)	1.29 (2.90)	0.27	1.31	2.28	6.37
	40-49	155	51	0.62 (3.37)	5.67 (63.75)	0.77	5.78	2.22	17.03
	50-59	317	28	0.72 (14.63)	7.36 (91.42)	0.89	7.51	1.70	15.38
	60-69	94	6	0.71 (1.84)	491 (3399.77)	0.88	50.19	1.09	52.19
	70-79	25	1	2.32 (19.73)	17.00 (NA)	2.86	17.35	2.22	14.16

Sampling area (reference number)	Length class (cm)	No. of fish		Mean abundance (variance)		Standardized mean abundance		Standardized mean density	
		1985-87	2012-13	1985-87	2012-13	1985-87	2012-13	1985-87	2012-13
	80-89	19	1	7.89 (547.77)	7.00 (NA)	9.74	7.15	4.93	4.11
	≥ 90	32	0	21.97 (904.61)	NA (NA)	27.12	NA	6.41	NA
	Total	660	94	NA (NA)	8.76 (359.93)	NA	8.94	NA	20.40

Table 3.9 Sample sizes, standardized abundance and density (Standardized numbers were calculated by multiplying the mean abundance or density by 1/detection rate based on respective detection rates) of *Pseudoterranova decipiens* s.l. in the musculature of Atlantic cod (*Gadus morhua*) sampled from various areas surrounding Newfoundland and Labrador, and on the Flemish Cap during 1947-1953 (Templeman et al. 1957) and 2012-2013. Sampling areas identified below correspond to those described in Figure 11 and Table IV of Templeman et al. (1957; Appendices C and D), and reference numbers correspond to Fig. 2.2. This table includes only fish ≥ 41 cm and only data for the fillets (napes were excluded).

Sampling area – NAFO Division (reference number)	No. of fish		Standardized abundance		*Standardized density	
	1947-53	2012-13	1947-53	2012-13	1947-53	2012-13
NE coast Nfld - offshore 3K (1)	699	69	0.02	0.19	0.04	0.25
N Grand Bank - 3L W of long 50°W (2)	1004	33	0.02	0.25	0.02	0.29
Flemish Cap - 3M (3)	357	80	0.00	0.03	0.00	0.03
SE Grand Bank - 3N (4)	1550	94	0.01	0.05	0.01	0.06
SW Grand Bank - 3O (5)	922	35	0.01	0.26	0.01	0.60

Sampling area – NAFO Division (reference number)	No. of fish		Standardized abundance		*Standardized density	
	1947-53	2012-13	1947-53	2012-13	1947-53	2012-13
St. Pierre Bank S of lat 46°30’N - 3P (6)	344	150	0.07	0.39	0.13	0.79
S coast Nfld, Cape St. Mary's to Pass Island - 3P (7)	736	41	0.23	2.07	0.29	4.62
S coast Nfld, Pass Island to Cape Ray - 3P (8)	935	86	1.19	6.26	1.72	17.92
S section of Nfld W coast, S of Portland Creek - 4R (9)	599	47	1.16	2.85	1.40	4.42

* Density is calculated as the total nematodes/kg from all fish combined. This was done to adhere to Templeman et al. (1957) as raw data were not available.

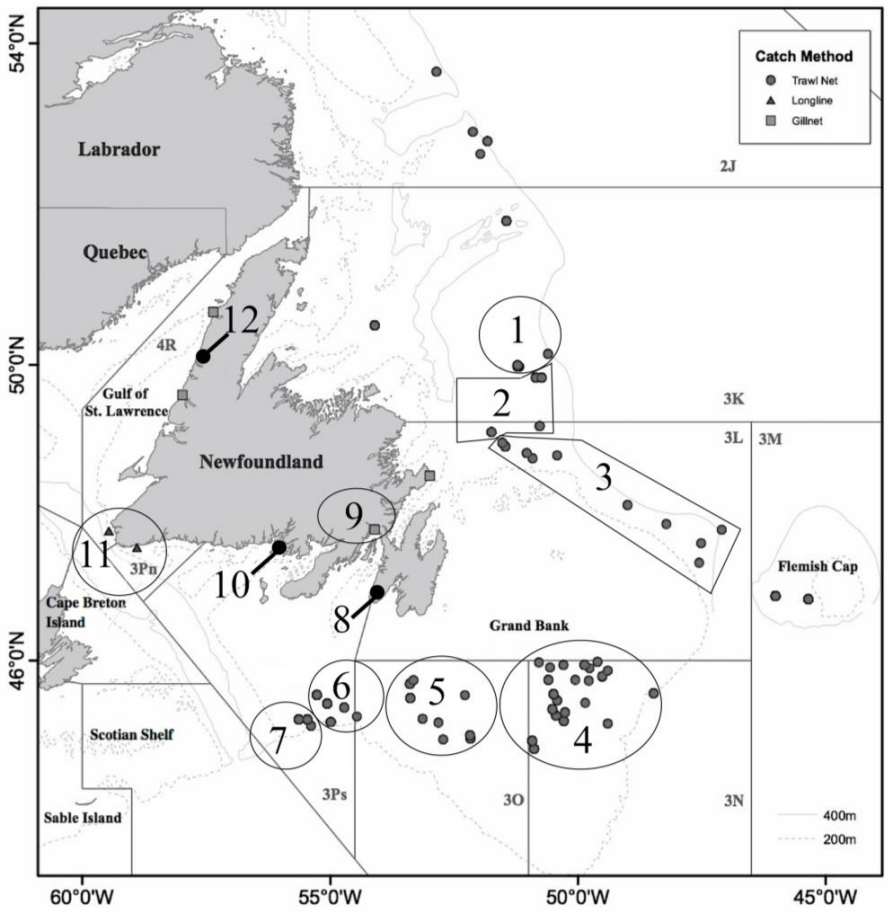

Figure 3.1 Study area indicating NAFO Division boundaries and locations where Atlantic cod (*Gadus morhua*) were sampled during 2012-2013. Numbers surrounded by shapes indicate where samples were grouped for the comparison of nematode infection levels corresponding to Figure 1 of Brattey et al. (1990; Appendix B). 1 = Southern Funk Island Bank, 2 = Northeast Newfoundland Shelf, 3 = Grand Bank, 4 = Southeast Shoal of the Grand Bank, 5 = Whale Bank, 6 = Green Bank, 7 = Southern St. Pierre Bank, 8 = Cape St. Mary's, 9 = Placentia Bay, 10 = Pass Island, 11 = Rose Blanche Bank, 12 = Portland Creek. Each point indicates a location where multiple fish were collected (Appendix A). Any samples not encircled were not used in the comparison to these historical data from Brattey et al. (1990).

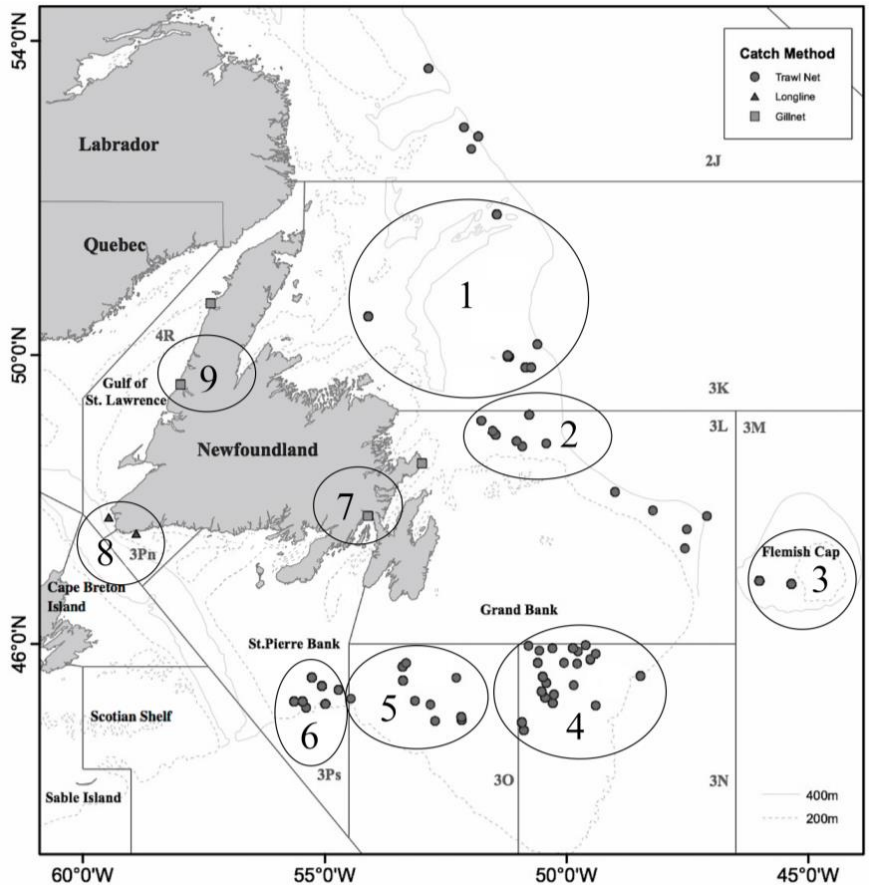

Figure 3.2 Study area indicating NAFO Division boundaries and locations where Atlantic cod (*Gadus morhua*) were sampled during 2012-2013. Numbers surrounded by shapes indicate where samples were grouped for the comparison of nematode infection levels corresponding to Figure 11 and Table IV of Templeman et al. (1957; Appendices C and D). 1 = Northeast Coast of Newfoundland, 2 = Northern Grand Bank (west of long 50° W), 3 = Flemish Cap, 4 = Southeast Grand Bank, 5 = Southwest Grand Bank, 6 = St. Pierre Bank (south of lat 46°30' N), 7 = Cape St. Mary's to Pass Island, 8 = Pass Island to Cape Ray, 9 = South of Portland Creek. Each point indicates a set where multiple fish were collected (Appendix A). Any samples not encircled were not used in the comparison to these historical data from Templeman et al. (1957).

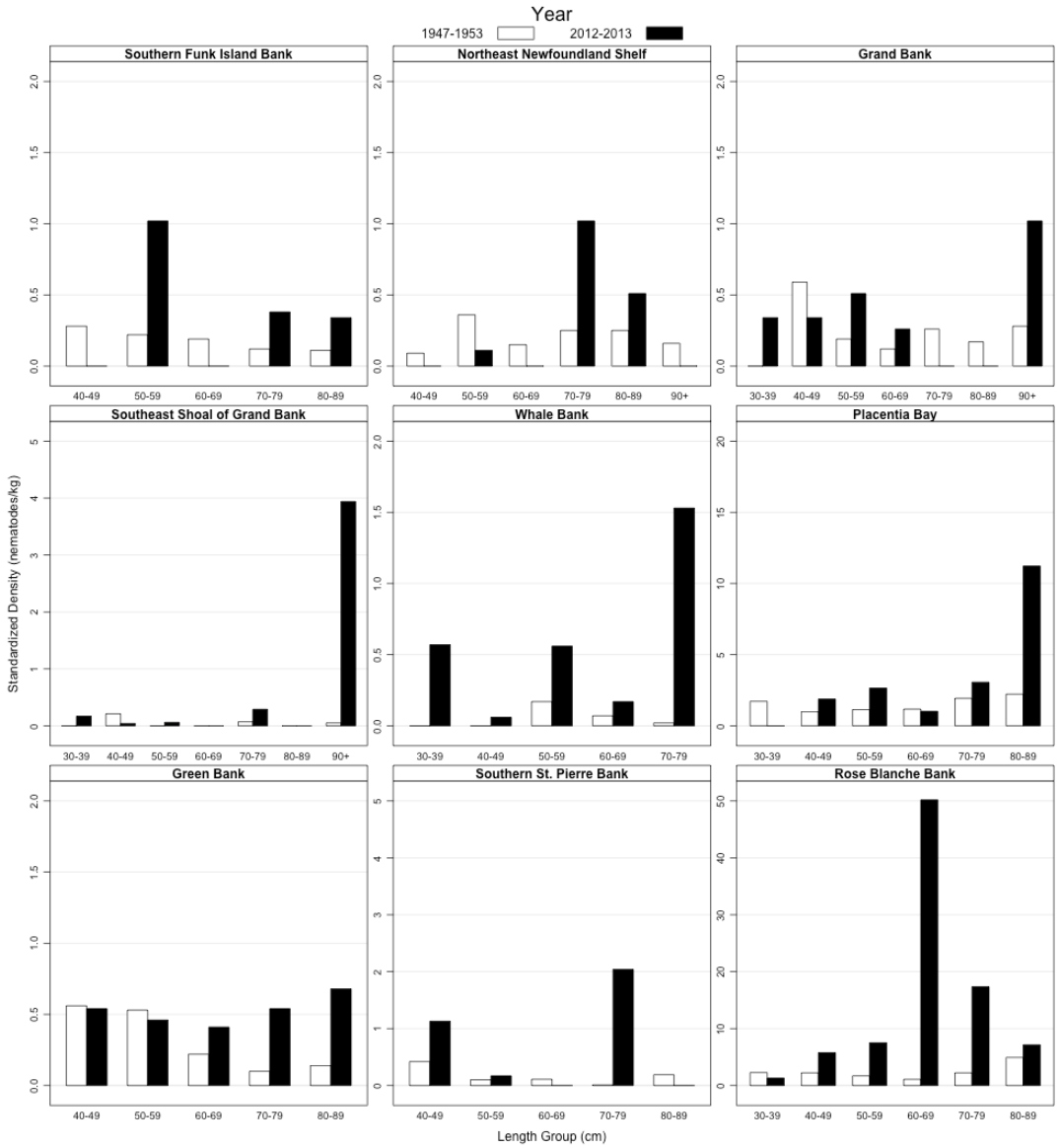

Figure 3.3 Mean standardized density (nematodes/kg of musculature) of *Pseudoterranova decipiens* s.l. found in the musculature of Atlantic cod (*Gadus morhua*) from various areas off Newfoundland and Labrador during 1985-1987 (Brattey et al. 1990) and 2012-2013. Note the change in scale for each panel. Standardized numbers were calculated by multiplying the mean density by 1/detection rate, based on respective detection rates.

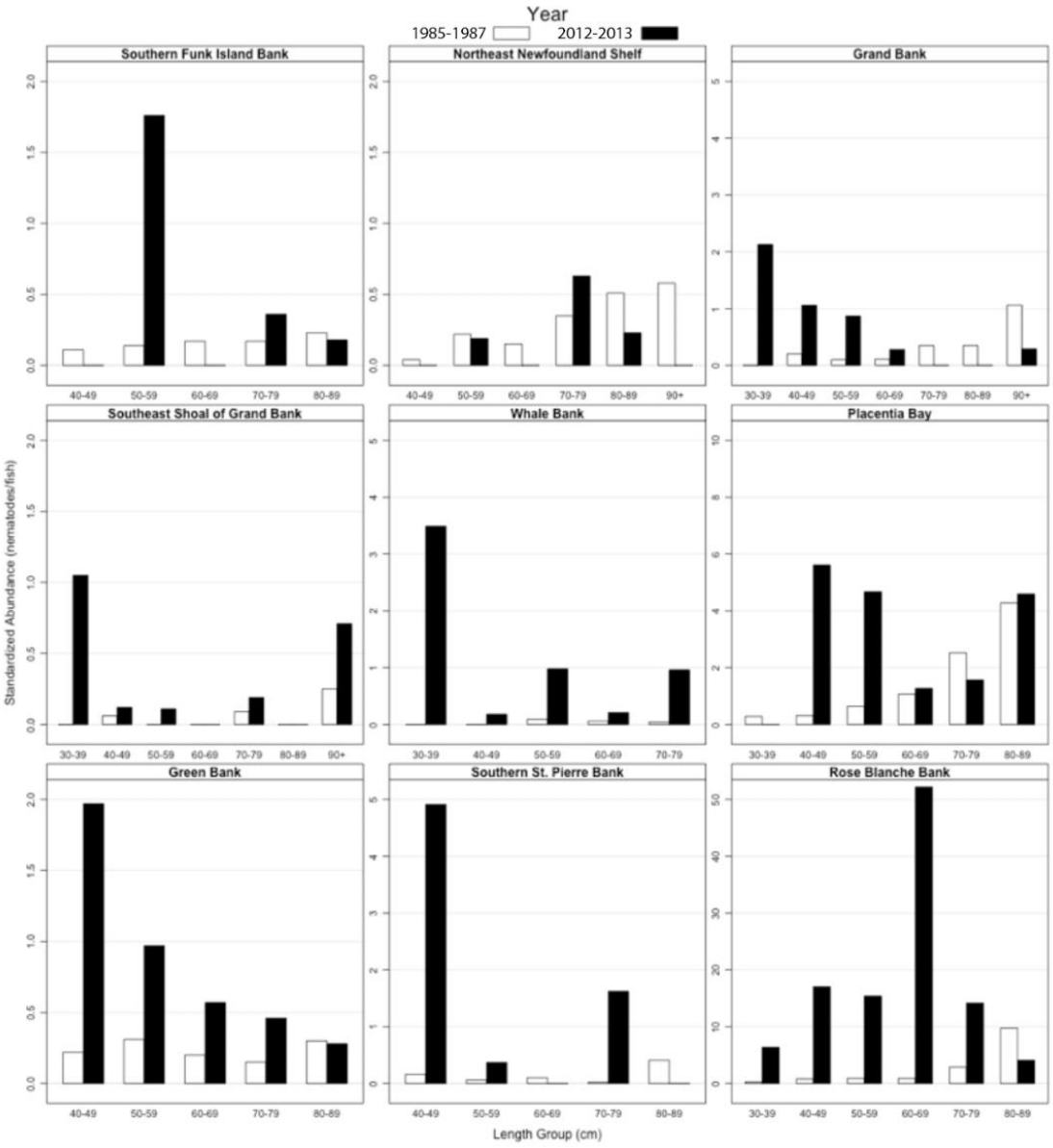

Figure 3.4 Mean standardized abundance (nematodes/fish) of *Pseudoterranova decipiens* s.l. in the musculature of Atlantic cod (*Gadus morhua*) from various areas off Newfoundland and Labrador during 1985-1987 (Brattey et al. 1990) and 2012-2013. Note the change in scale for each panel. Standardized numbers were calculated by multiplying the mean density by 1/detection rate, based on respective detection rates.

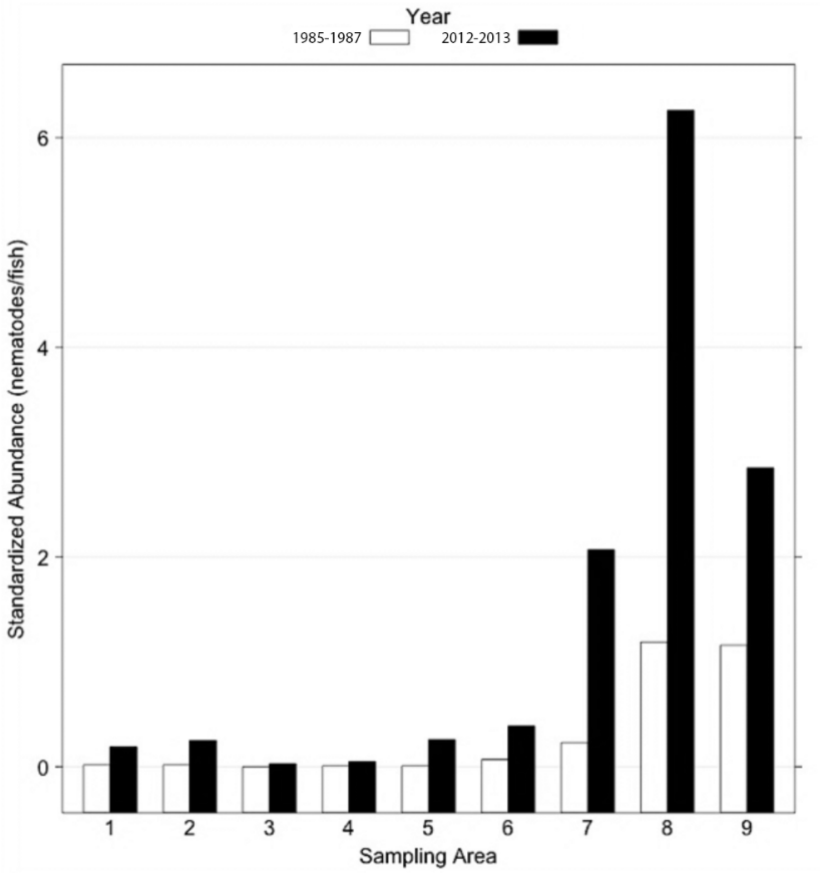

Figure 3.5 Mean standardized abundance (nematodes/fish) of *Pseudoterranova decipiens* s.l. in the musculature of Atlantic cod (*Gadus morhua*) sampled from various areas off Newfoundland and Labrador, and on the Flemish Cap during 1947-1953 (Templeman et al. 1957) and 2012-2013. Only Atlantic cod ≥ 41 cm and only nematodes found in the fillets (not the napes) were included in these analyses. 1 = Northeast Coast of Newfoundland, 2 = Northern Grand Bank (west of long 50° W), 3 = Flemish Cap, 4 = Southeast Grand Bank, 5 = Southwest Grand Bank, 6 = St. Pierre Bank (South of lat 46°30’ N), 7 = Cape St. Mary’s to Pass Island, 8 = Pass Island to Cape Ray, 9 = South of Portland Creek. Standardized numbers were calculated by multiplying the mean density by 1/detection rate, based on respective detection rates.

4 General Discussion and Conclusion

With some Atlantic cod stocks off Newfoundland and Labrador showing signs of improvement in recent years (Rose and Rowe 2015; DFO 2016; DFO 2017a), there has been a renewed interest in commercial fishing, evoking a need to update and quantify the infection levels of anisakid nematodes found in the musculature of Atlantic cod. By following a similar methodology to Brattey et al. (1990) and Brattey and Bishop (1992), the musculature of 811 Atlantic cod collected during 2012-2013 were examined for two larval parasitic nematodes, *Anisakis simplex* s.l. and *Pseudoterranova decipiens* s.l.

For the first component of my study, I examined the geographic distribution of larval *A. simplex* s.l. and *P. decipiens* s.l. within the musculature of Atlantic cod from five stocks off Newfoundland and Labrador, and on the Flemish Cap. Both *A. simplex* s.l. and *P. decipiens* s.l. are widespread in the Northwest Atlantic, however they differ greatly in terms of where the highest abundances are located. Atlantic cod collected on the Flemish Cap harboured the highest intensities of *A. simplex* s.l., and the northeast coast of Newfoundland and the southern Grand Bank had moderate levels, which may be due to the distribution of cetaceans in these areas (Marcogliese 1995). However, without better information on trends in cetacean population size and distribution, as well as their level of infection with reproductive adults of *A. simplex* s.l., it remains difficult to determine whether this geographic distribution in infection levels of larval *A. simplex* s.l. in cod were driven by population changes among cetacean definitive hosts or other factors. The positive relationship between *P. decipiens* s.l. and its main definitive host, the grey seal,

is much more apparent. The significant increase in grey seals in eastern Canada coincides with the geographic distribution of *P. decipiens* s.l. on the west and south coasts of Newfoundland. Overall, the Newfoundland cod stocks that are located farther offshore and eastward have the lowest infection levels of *P. decipiens* s.l., although they also have the highest infection levels of *A. simplex* s.l.

The second component of this thesis is to examine the distribution of nematodes in the musculature and whether they were concentrated in the left or right sides of the nape or fillet. There were many differences in the concentration of nematodes between *A. simplex* s.l. and *P. decipiens* s.l. *Anisakis simplex* s.l. nematodes were found to be highly concentrated in the napes, of these, significantly more were found in the left side of the fish with significant heterogeneity among size groups. A small percentage of nematodes were observed in the fillet, and were not found to be more predominant in either the left or right side of the fish. The distribution of *P. decipiens* s.l. on the other hand was much more dependent on the length of fish. Smaller individuals had many more nematodes encysted in the fillets as opposed to the napes proportionally, whereas larger individuals had nematodes more evenly distributed between the nape and fillet, which is illustrated by the significant heterogeneity found among length groups. There were also more nematodes found in the left side of the fish in both the nape and fillet, though the percent in the left side of the nape appeared to decrease with fish length. Significant heterogeneity was also found for nematodes located in the left versus right side of the fish in the nape but not the fillet.

Another trend observed was that infection levels of *A. simplex* s.l. and *P. decipiens* s.l. tended to increase in relation to the length of the fish. As cod grow, they shift from eating

invertebrate euphausiid prey items to a more piscivorous diet, which includes fish species known to harbour multiple infections of both *A. simplex* s.l. and *P. decipiens* s.l. larvae (Marcogliese 1995; Martell and McClelland 1995; Link and Garrison 2002; Sherwood et al. 2007). Therefore, larger and older cod ingest much greater numbers of larval nematodes with more heavily infected prey, thus increasing the likelihood of multiple infections.

The final aspect of this thesis assesses whether infection levels of *A. simplex* s.l. and *P. decipiens* s.l. in the musculature of cod have fluctuated since the surveys conducted from 1947-1953 (Templeman et al. 1957) and 1983-1985 (Brattey et al. 1990; Brattey and Bishop 1992). Although a statistical approach could not be taken due to unreported variances in the case of Templeman et al. (1957), as well as differences in the rate of nematode detection by candling, the data suggest an increasing trend of *A. simplex* s.l. infection levels in all areas investigated since the 1947-1953, with a more rapid increase observed during the last three decades. The cod sampled on the Flemish Cap in particular had a substantial increase in the infection levels of *A. simplex* s.l., and the same was observed on the west and south coasts of Newfoundland. A total of 34 cetaceans were found to harbour *A. simplex* s.l. (Klimpel and Palm 2011) and as previously considered, an increase in abundance of some of these cetaceans (the main definitive hosts of *A. simplex* s.l.) may offer an explanation for the increasing infection levels of *A. simplex* s.l. in the musculature of Atlantic cod (Arneberg 2001), however population trends of most cetaceans are not well known. In 2007, a large aerial survey was conducted in the Northwest Atlantic to survey marine megafauna in order to quantify their abundance (Lawson and Gosselin 2009). Although this has expanded the knowledge of

cetacean populations, there is still uncertainty remaining as to which species might be responsible for contributing large numbers of *A. simplex* s.l. into the environment.

The infection levels of *P. decipiens* s.l. in the musculature of cod also appeared to have increased slightly in most areas since the historical surveys conducted, but increased more noticeably in the inshore areas, particularly the west and southwest coasts of Newfoundland. This is consistent with the increasing population of grey seals, the main definitive host on the east coast of Canada (DFO 2017b). There have been substantial increases in harp seal populations, however a decline in their population has been observed in Gulf of St. Lawrence where the most marked increases in *P. decipiens* s.l. were seen (DFO 2012). Though the overall increase may partially contribute to the increased levels of *P. decipiens* s.l. found in cod, they are not considered important hosts (Marcogliese et al. 1996). The impact of harbour seals, which have been observed to carry substantial numbers of *P. decipiens* s.l. (Brattey and Stenson 1993), on infection in cod cannot be assessed since little is known about their status off the east coast of Canada; they may be locally abundant, but overall population size is much less than that of grey seals.

Although changes in the distribution and abundance of the definitive hosts of these nematodes may be contributing factors, many other factors may be influencing the increasing infection levels observed in this study. In parasitological surveys conducted in the Gulf of St. Lawrence (Boily and Marcogliese 1995; Marcogliese et al. 1996), the decreasing trend in *P. decipiens* s.l. infection in fishes was attributed to the decreasing bottom temperatures which inhibited the hatching and development of the eggs. Conversely, warming trends in the Atlantic Ocean accelerate the hatching times,

development and survival of *P. decipiens* s.l. (McClelland et al. 2011) and *A. simplex* s.l. (Brattey and Clark 1992). Increasing bottom temperatures have also been recorded in northern areas and on the Flemish Cap (Colbourne et al. 2014). The warming trend seen over the past few years in the North Atlantic (Colbourne et al. 2014) may partly explain the increased levels of *A. simplex* s.l. and *P. decipiens* s.l. infection in the flesh of cod. Should this warming trend continue, it is likely that there will continue to be an increase in the abundance and prevalence of larval anisakine nematodes found in not only Atlantic cod, but any species that may become host to these parasites.

Many parasitological studies conducted worldwide have also suggested similar factors influencing an increase in nematode abundance. A study in Iceland concluded that Atlantic cod caught inshore near the definitive host, the grey seal, haul out sites which coincided with warmer waters than offshore areas, had much higher levels of *P. decipiens* s.l. infection (Hauksson 2011). Similarly, McClelland et al. (2000) suggested that increased abundance of *P. decipiens* s.l. in American plaice was correlated to high near-bottom temperatures as well as proximity to the definitive host.

The diet of Atlantic cod is quite varied (Fahay et al. 1999), and a shift in this diet is to be expected with major changes that have occurred in the Northwest Atlantic ecosystems during recent decades (Worm and Myers 2003; Frank et al. 2005). Increased infection levels of *A. simplex* s.l. and *P. decipiens* s.l., which are transmitted to Atlantic cod through predator-prey interactions by various invertebrates and fish hosts, could be a result of a diet shift to species that are more heavily infected by these parasites. Infection levels of *A. simplex* s.l. larvae in euphausiids are typically very low, and therefore cod likely acquire these nematodes individually (Hays et al. 1998a). However, an increase in

certain fishes, such as Atlantic herring and American plaice, has been observed in the diet of cod in the Gulf of St. Lawrence (Hanson and Chouinard 2002). Atlantic herring is known to harbour substantial abundances of *A. simplex* s.l. (Hays et al. 1998b), whereas *P. decipiens* s.l. infects American plaice particularly in the Gulf of St. Lawrence (Boily and Marcogliese 1995). An increase in these prey items in the diet of Atlantic cod would likely increase the number of nematodes they are exposed to and cause an increase in infection levels. Spatial differences in diet would also influence the number of nematodes cod are exposed to. For example, the diet of northern cod around Newfoundland and Labrador is primarily benthic, containing almost exclusively northern shrimp, which are not known to harbour anisakine nematodes (Caddy 1989). However, the diets of cod farther south contain more pelagic fishes, based on stomach contents and stable carbon isotopes (Sherwood et al. 2007). Unfortunately, without a better understanding of which species are involved in the life cycle of both *A. simplex* s.l. and *P. decipiens* s.l., it remains difficult to determine how a change in diet would influence abundance of these anisakine nematodes. Further research focusing on the life cycle of both *A. simplex* s.l. and *P. decipiens* s.l. would be beneficial in understanding the geographic patterns, as well as temporal changes in infection levels for these nematodes.

4.1 References

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Appendix A. Sampling details for Atlantic cod (*Gadus morhua)* collected off Newfoundland and Labrador, and on the Flemish Cap during 2012-2013 and examined for nematodes. B. ref indicates reference numbers corresponding to sampling areas described in Figure 1 of Brattey and Bishop (1992) to which that set was assigned (Appendix B). T. ref indicates reference numbers corresponding to sampling areas described in Table IV and illustrated in Figure 11 of Templeman et al. (1957; Appendices C and D) to which that set was assigned.

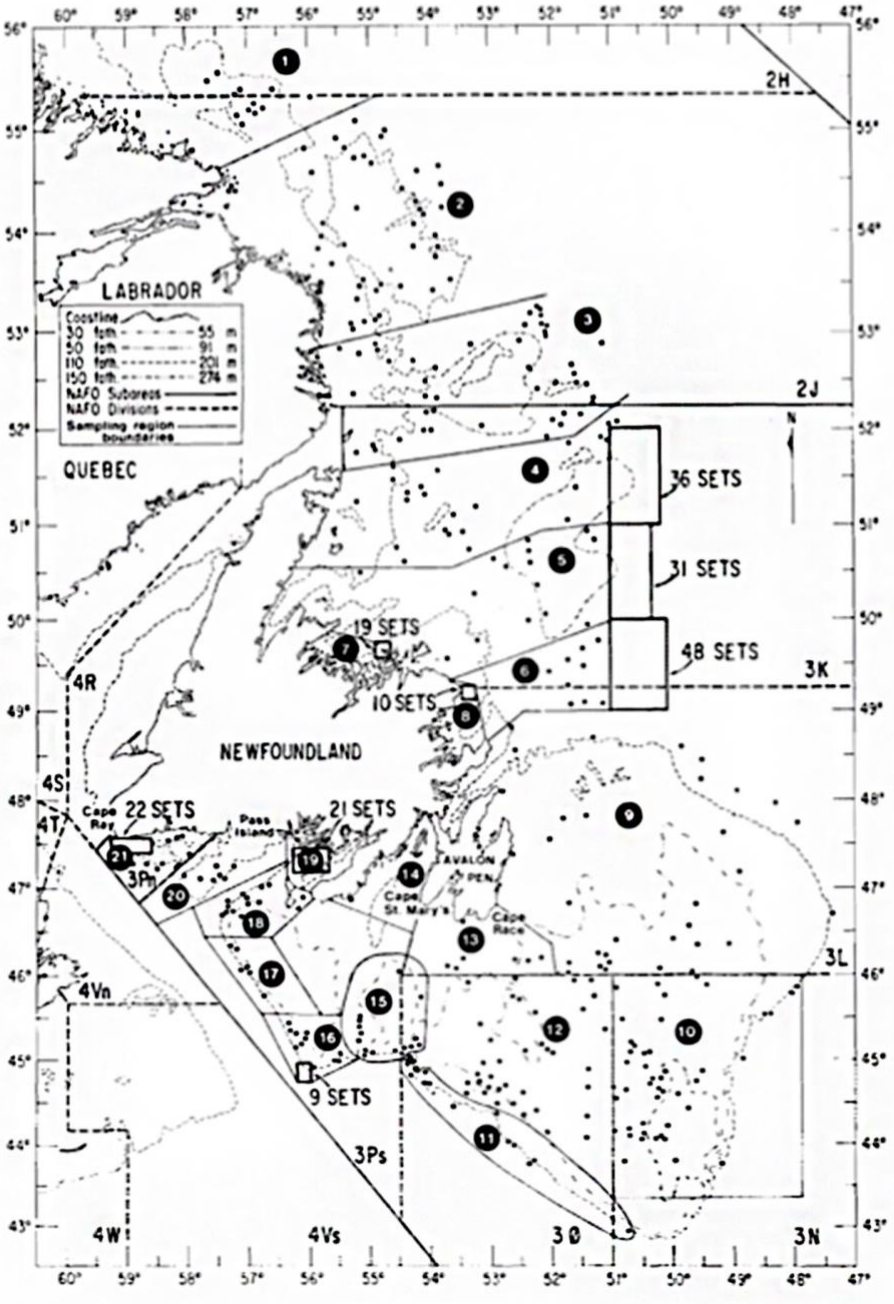
Date	NAFO Div.	Latitude (N)	Longitude (W)	Gear type	No. of fish	B. ref	T. ref
29 Apr. 2013	2J	53.6633	-52.8500	trawl	3	2	8
1 May 2013	2J	52.6683	-51.9617	trawl	1	3	8
1 May 2013	2J	52.8200	-51.8217	trawl	10	3	8
30 Apr. 2013	2J	52.9367	-52.1117	trawl	2	3	8
4 Dec. 2012	3K	50.5150	-54.0917	trawl	9	4	6
1 May 2013	3K	51.8383	-51.4350	trawl	14	4	6
9 May 2013	3K	49.9743	-51.1780	trawl	10	5	6
8 May 2013	3K	49.9822	-51.2150	trawl	11	5	6
8 May 2013	3K	49.9970	-51.2132	trawl	19	5	6
3 May 2013	3K	50.1483	-50.5967	trawl	2	5	6
10 May 2013	3L	49.0098	-51.7275	trawl	9	6	3
3 Dec. 2012	3L	49.1967	-50.7633	trawl	8	6	3
4 May 2013	3K	49.8352	-50.8437	trawl	11	6	6

Date	NAFO Div.	Latitude (N)	Longitude (W)	Gear type	No. of fish	B. ref	T. ref
4 May 2013	3K	49.8378	-50.7278	trawl	4	6	6
30 Jul. 2012	3L	48.5333	-52.9833	trawl	12	8	9
2 Dec. 2012	3L	48.7750	-50.9100	trawl	2	9	3
2 Dec. 2012	3L	48.8117	-50.4183	trawl	2	9	3
2 Dec. 2012	3L	48.8433	-51.0267	trawl	4	9	3
26 Nov. 2012	3L	48.9300	-51.4617	trawl	7	9	3
26 Nov. 2012	3L	48.9767	-51.5267	trawl	14	9	3
19Nov. 2012	3L	47.3600	-47.5517	trawl	1	9	4
20 Nov. 2012	3L	47.6267	-47.5067	trawl	2	9	4
20 Nov. 2012	3L	47.8117	-47.0917	trawl	3	9	4
22 Nov. 2012	3L	47.8900	-48.2150	trawl	1	9	4
22 Nov. 2012	3L	48.1467	-48.9967	trawl	1	9	4
19 Oct. 2012	3N	44.7417	-50.8767	trawl	15	10	2
30 Sep. 2012	3N	44.8617	-50.9183	trawl	35	10	2
2 Jun. 2012	3N	45.1050	-49.3967	trawl	3	10	2
1 Jun. 2012	3N	45.1467	-50.2817	trawl	4	10	2
1 Jun. 2012	3N	45.2250	-50.4417	trawl	3	10	2
1 Jun. 2012	3N	45.2733	-50.2583	trawl	3	10	2
1 Jun. 2012	3N	45.3150	-50.5117	trawl	11	10	2
11 Oct. 2012	3N	45.4033	-49.8467	trawl	1	10	2

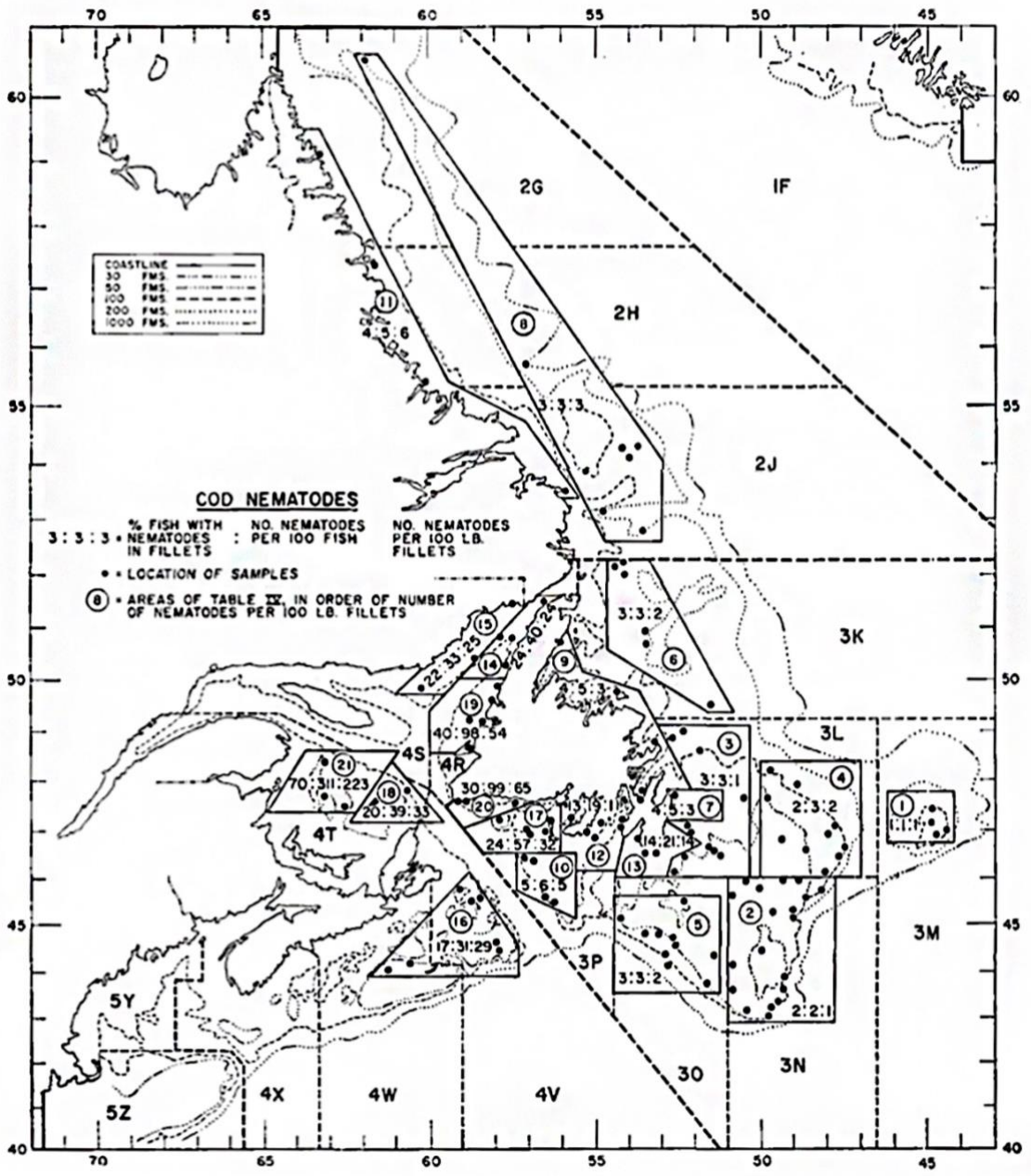
Date	NAFO Div.	Latitude (N)	Longitude (W)	Gear type	No. of fish	B. ref	T. ref
1 Jun. 2012	3N	45.4367	-50.4150	trawl	6	10	2
1 Jun. 2012	3N	45.5267	-50.4850	trawl	9	10	2
3 Jun. 2012	3N	45.5433	-48.4667	trawl	6	10	2
2 Jun. 2012	3N	45.7200	-49.7750	trawl	1	10	2
11 Oct. 12	3N	45.7300	-50.5900	trawl	4	10	2
2 Jun. 2012	3N	45.7317	-50.0483	trawl	1	10	2
11 Oct. 2012	3N	45.7717	-49.5050	trawl	1	10	2
11 Oct. 2012	3N	45.7800	-49.5083	trawl	3	10	2
11 Oct. 2012	3N	45.8600	-49.3950	trawl	2	10	2
2 Jun. 2012	3N	45.8933	-49.7600	trawl	1	10	2
1 Jun. 2012	3N	45.9017	-50.5600	trawl	2	10	2
11 Oct. 2012	3N	45.9383	-50.2850	trawl	4	10	2
11 Oct. 2012	3N	45.9433	-49.8600	trawl	5	10	2
1 Jun. 2012	3N	45.9767	-50.7800	trawl	1	10	2
11 Oct. 2012	3N	45.9817	-49.6017	trawl	4	10	2
1 Oct. 2012	3O	44.8817	-52.7150	trawl	3	12	5
1 Oct. 2012	3O	44.8950	-52.1633	trawl	10	12	5
1 Oct. 2012	3O	44.9433	-52.1700	trawl	10	12	5
1 Oct. 2012	3O	45.1233	-52.8117	trawl	1	12	5
1 Oct. 2012	3O	45.1767	-53.1250	trawl	3	12	5

Date	NAFO Div.	Latitude (N)	Longitude (W)	Gear type	No. of fish	B. ref	T. ref
30 Sep. 2012	3O	45.4750	-53.3783	trawl	10	12	5
1 Oct. 2012	3O	45.5117	-52.2733	trawl	1	12	5
30 Sep. 2012	3O	45.6733	-53.3800	trawl	16	12	5
30 Sep. 2012	3O	45.7267	-53.3150	trawl	3	12	5
16 Sep. 2013	3Ps	47.8167	-54.1000	gillnet	43	14	12
2 Oct. 2012	3O	45.2100	-54.4517	trawl	1	15	5
18 May 2013	3Ps	45.1307	-54.9793	trawl	60	15	10
16 May 2013	3Ps	45.3358	-54.7120	trawl	5	15	10
16 May 2013	3Ps	45.3967	-55.0552	trawl	23	15	10
21 May 2012	3Ps	45.5168	-55.2613	trawl	48	15	10
18 May 2013	3Ps	45.0815	-55.3802	trawl	3	16	10
17 May 2013	3Ps	45.1670	-55.6283	trawl	13	16	10
17 May 2013	3Ps	45.1673	-55.4527	trawl	12	16	10
22 Jul. 13	3Pn	47.5687	-58.8932	longline	61	21	20
26 Jul. 13	4R	47.7947	-59.4545	longline	33	21	20
6 May. 12	3M	46.8592	-45.3463	trawl	38	NA	1
24 Apr. 2013	3M	46.9020	-46.0090	trawl	67	NA	1
12 Oct. 2012	4R	50.6800	-57.3467	gillnet	13	NA	14
29 Jul. 2013	4R	49.6090	-57.9752	gillnet	47	NA	19

Appendix B. Figure 1 extracted from Brattey and Bishop (1992) illustrating where samples of Atlantic cod (*Gadus morhua*) were collected. Sampling areas in the current study were grouped as closely as possible to regions described below in order to compare infection levels of *Anisakis simplex* s.l. and *Pseudoterranova decipiens* s.l. (Appendix A).



Appendix C. Figure 11 extracted from Templeman et al. (1957) illustrating where samples of Atlantic cod (*Gadus morhua*) were collected. Sampling areas in the current study were grouped as closely as possible to regions described below in order to compare infection levels of *Anisakis simplex* s.l. and *Pseudoterranova decipiens* s.l. (Appendix A).



Appendix D. Table IV extracted from Templeman et al. (1957) describing localities illustrated in Figure 11 (Templeman et al. 1957).

TABLE IV. Nematode infection in cod of commercial size, 41 cm. and over, as determined by destructive examination of the fillets.

Reference No.	Locality and ICNAF subdivision	No. cod	Gutted weight	Percentage of cod with nematodes in fillets	No. of nematodes per 100 cod	No. of nematodes per 100 lb. of fillets	No. of nematodes identified	Percentage *Porrocaecum*	Percentage *Anisakis*
			lb.	%				%	%
1	Flemish Cap: 3M	357	1,517	1.4	1.4	1.0	3	0	100
2	SE. Grand Bank: 3N	1,550	8,717	1.9	1.9	1.0	7	43	57
3	N. Grand Bank: 3L[1] W. of long. 50° W.	1,004	6,397	2.5	2.6	1.2	8	75	25
4	N. Grand Bank: 3L[2] E. of long. 50° W.	1,196	6,529	2.2	2.7	1.5	16	75	25
5	SW. Grand Bank: 3O	922	4,036	2.5	2.8	1.9	14	29	71
6	NE. coast Nfld.—offshore: 3K	699	2,990	3.0	3.0	2.1	3	67	33
7	St. John's: 3L[1]	1,611	7,232	3.7	4.7	3.1	31	84	16
8	Labrador—offshore: 2G, 2H, 2J	851	2,760	3.1	3.4	3.2	11	36	64
9	E. coast Nfld. inshore: 3K, 3L[1]	1,089	4,545	3.8	4.6	3.3	14	100	0
10	St. Pierre Bank south of lat. 46°30′ N.: 3P	344	1,159	4.9	5.8	5.2	16	94	6
11	Labrador—inshore: 2G, 2H, 2J	880	2,191	4.3	5.1	6.2	16	94	6
12	S. coast Nfld.—Cape St. Mary's to Pass Island: 3P	736	3,863	12.5	18.9	10.8	97	97	3
13	Cape Race to Cape St. Mary's: 3L[1]	257	1,152	14.0	21.4	14.3	7	57	43
14	N. section of Nfld. W. Coast—N. of Portland Creek: 4R	629	3,079	23.9	39.8	24.4	105	92	8
15	N. Gulf of St. Lawrence: 4S	259	1,013	22.4	32.8	25.2	50	84	16
16	Nova Scotia banks: 4V, 4W	553	1,789	17.0	31.1	28.9	101	98	2
17	St. Pierre Bank north of lat. 46°30′ N. and Burgeo Bank: 3P	551	2,923	24.3	57.0	32.2	109	92	8
18	S. Gulf of St. Lawrence (deeper water, 51–90 fath.): 4T	169	595	19.5	39.1	33.3	28	100	0
19	S. section of Nfld. W. coast—S. of Portland Creek: 4R	599	3,288	39.7	98.1	53.6	190	96	4
20	S. coast Nfld.—Pass Island to Cape Ray: 3P	935	4,260	30.4	99.1	65.3	362	97	3
21	S. Gulf of St. Lawrence—(shallow water, 26–50 fath.): 4T	267	1,113	70.0	310.5	223.2	399	100	0
Total		15,458	71,148	10.5	24.5	16.0	1,587	94.3	5.7